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**Risk assessment of Shiga-toxin producing
Escherichia coli O157 in steak tartare in the
Netherlands**

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Abstract

The methodology of quantitative microbiological risk assessment (QMRA), a tool to evaluate food related health risks, is rapidly developing. As a contribution to this development, a QMRA of Shiga-toxin producing *E. coli* (STEC) O157 in steak tartare in the Netherlands is conducted, using the Modular Process Risk Model (MPRM) concept. STEC O157 has caused a number of large-scale outbreaks in several industrial countries with severe public health consequences, often associated with the consumption of beef products. An exposure model was constructed, covering the whole food pathway from farm animals to human consumption. This model was linked with a newly developed dose response model of STEC O157 based on Japanese outbreak data. It resulted in estimates of steak tartare contamination (prevalence and concentration) and the incidence of STEC O157 associated illness by steak tartare consumption. As in other QMRAs, these estimates are highly uncertain as a consequence of a lack of adequate data all over the food pathway. Expert opinion was used to obtain estimates of several model parameters. Compared with independent data, the model estimate of the prevalence of contaminated raw tartare patties (0.3%) is low, whereas the estimated incidence rate of diarrhoeal illness (8 per 100.000 person years) is high. The QMRA approach allows for an overall scenario analysis. It was found that intervention at the farm or during slaughter is probably more efficient to reduce STECO157 health risks than intervention at the consumer stage. Furthermore, important data gaps could be identified.

Preface

The risk assessment described in this report is the result of extensive collaboration between the authors and a variety of researchers and experts in different disciplines. We are grateful that those researchers and experts were prepared to expose themselves to our -sometimes unusual- questions and provide us -if possible- with answers and data. We would also like to thank several colleagues who critically reviewed (part of) the report.

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Samenvatting

In toenemende mate wordt gebruik gemaakt van kwantitatieve microbiologische risicoschatting (Quantitative microbiological risk assessment, QMRA) om voedselgerelateerde microbiële gezondheidsrisico's te beheersen. Onlangs is het 'Modular Process Risk Model' (MPRM) gelanceerd als methode waarmee een risicomodel 'van boerderij tot consument' kan worden opgezet. De transmissie van een microbiologisch gevaar over het hele traject kan er kwantitatief mee worden beschreven. Bestaande gezondheidsrisico's kunnen worden ingeschat, en risicomangers wordt de mogelijkheid geboden om verschillende opties voor interventie ter verlaging van de risico's modelmatig door te rekenen en te vergelijken.

QMRA is een complex onderzoeksgebied en nog volop in ontwikkeling. De doelstelling van het in dit rapport beschreven onderzoek was daarom voornamelijk om meer ervaring op te doen in het uitvoeren van een QMRA 'van boerderij tot volksgezondheid'. Blootstellingschatting van de consument is gecombineerd met dosis-respons modellering. Het microbiologische gevaar dat onderzocht werd is Shiga-toxine producerende *Escherichia coli* O157 (STEC O157), een beruchte pathogeen. Infectie met STEC O157 kan leiden tot verschillende symptomen, variërend van milde gastro-enteritis, haemorrhagische colitis, het haemolytisch uremisch syndroom (HUS) bij jonge kinderen, tot sterfte. Ernstige explosies van STEC O157 zijn tot dusver niet in Nederland voorgekomen, maar uit verschillende andere (westerse) landen zijn deze wel bekend. Rundvee wordt veelal beschouwd als het belangrijkste reservoir van STEC O157. Het levensmiddel dat in deze risicoschatting bestudeerd werd is daarom een rundvleesproduct. De keuze is gevallen op rundertartaar, vanwege de consumptiefrequentie, het potentiële risico (omdat rundertartaar (deels) rauw gegeten wordt) en de relatieve eenvoud van het productieproces. Het onderzoek beperkte zich verder tot Nederlandse runderen en slachthuizen, en Nederlandse consumenten.

Volgens de richtlijnen van de MPRM methodiek is ten behoeve van de blootstellingschatting de proces-keten beschreven en opgedeeld in basisprocessen. Voor de basisprocessen 'opdelen' en 'mengen' zijn nieuwe modellen ontwikkeld waarmee de effecten van niet-willekeurige verdeling van micro-organismen in het substraat meegenomen kunnen worden.

Aangezien de wijze waarop runderen geslacht worden kan verschillen, is er onderscheid gemaakt tussen drie blootstellingsroutes. Deze routes verschillen in wijzen van slachten en verdere bewerking: 'industriële' en 'traditioneel'. Om aan te sluiten bij de effectmodellering, zijn consumenten ingedeeld in drie leeftijdsklassen: 1-4 jaar, 5-14 jaar and 15+. Daarnaast zijn er drie verschillende bereidingswijzen van tartaartjes meegenomen: rauw, licht gebakken en goed doorbakken. (Nederlandse) gegevens omtrent prevalentie en concentratie van STEC O157 zijn verzameld voor verschillende stadia van de keten: boerderij, slachterij, detailhandel en consument. Dit rapport biedt een uitgebreid overzicht van deze gegevens.

Bij het opstellen van het model bleek dat er weinig overlap is tussen de beschikbare gegevens en de gegevens die nodig zijn om de waarden van de modelparameters te schatten. Er is daarom een bijeenkomst met deskundigen georganiseerd, waarin de deskundigen is gevraagd de waarden van een aantal modelparameters in te schatten. Bij deze modelparameters valt

bijvoorbeeld te denken aan de fecale verontreiniging van karkassen uitgedrukt in gram feces per karkas. Het risicomodel is vervolgens geïmplementeerd in een @Risk spreadsheet waarmee Monte Carlo simulaties zijn uitgevoerd. Daarbij is alleen variabiliteit meegenomen, niet de onzekerheid. Vanwege de grote en complexe bronnen van onzekerheid, zowel in de proceskennis, de modellen en de gegevens, bleek het niet mogelijk de onzekerheid op een zinvolle manier te kwantificeren. Als alternatief is een 'standaardmodel' met de meest waarschijnlijke parameterwaarden vergeleken met alternatieve scenario's, modellen waarin de parameterwaarden zijn aangepast. Met deze methode kan het effect van onzekerheid in input-parameters op de output bestudeerd worden.

Uit de blootstellingschatting blijkt dat volgens het model ongeveer 0,3% van de rauwe tartaartjes besmet is met STEC O157. Het merendeel hiervan (>60%) is besmet met slechts één kolonievormende eenheid (kve). Hoge besmettingsniveaus zijn schaars. Vergelijking met een microbiologisch onderzoek waarin één op de 82 rauwe tartaartjes (1,2%) positief voor STEC O157 werd gevonden, wijst er op dat dit resultaat van het model een onderschatting van de werkelijkheid zou kunnen zijn.

Het blootstellingsmodel is gekoppeld aan een nieuw dosis-respons model voor STEC O157, dat is opgesteld met gegevens van een explosie op een Japanse basisschool in 1996. Van deze explosie zijn unieke humane gegevens bekend omtrent zowel de dosis als het aantal blootgestelde en geïnfecteerde personen. Zowel het exponentiële als hypergeometrische dosis-respons model zijn aan deze gegevens gefit. Zij voorspellen een hoge virulentie van STEC O157. Volgens het exponentiële model is de kans op ziekte per cel ongeveer 0,5%.

Het voorspelde aantal STEC O157 infecties in Nederland als gevolg van de consumptie van tartaar is volgens het 'standaardmodel' zo'n 2300, en het aantal ziektegevallen zo'n 1300. Dit laatste komt overeen met een incidentie van 8 per 100.000 persoonsjaren. Een onafhankelijke schatting van de totale incidentie in Nederland, op grond van epidemiologische gegevens, is 2000 ziektegevallen, of 13 per 100.000 persoonsjaren. Ogenscheinlijk wordt een groot deel van de STEC O157-gerelateerde ziektegevallen dus veroorzaakt door de consumptie van rundertartaar. De onzekerheid in de genoemde schattingen is echter groot. Een mogelijke overschatting van het aantal ziektegevallen is in tegenspraak met de mogelijke onderschatting waar het gaat om de besmetting van tartaartjes.

Deze conclusies blijven gelden als de onzekerheid in de parameterschattingen wordt meegenomen. Het blijkt dat de hoge ziekte-incidentie met name afhangt van het gebruikte dosis-respons model. Voorspellingen met andere dosis-respons modellen van STEC O157 vallen veel lager uit. Deze modellen zijn echter gebaseerd op niet humane gegevens of op andere pathogenen. Het is ook mogelijk dat de specifieke Japanse explosie niet representatief is voor de Nederlandse situatie met betrekking tot STEC O157 in tartaar, bijvoorbeeld omdat het in Japan een hoogvirulente STEC O157 betrof.

Ondanks de onzekerheid in de risicoschatting, kan het risicomodel gebruikt worden om de gevolgen van onzekerheid in verschillende processtappen te vergelijken, en om een uitspraak te doen over de effecten van interventie. Analyse van alternatieve scenario's laat zien dat de onzekerheid in prevalentie en concentratie van STEC O157 op de boerderij een groot effect kan hebben op het eindresultaat. Hetzelfde geldt voor groei en inactivatie van STEC O157 op

het karkas in het slachthuis. Het effect van groei van STEC O157 tijdens de opslag in de winkel en bij de consument thuis is daarentegen verwaarloosbaar, en het effect van voorlichting die moet leiden tot veiliger bereidingswijzen is twijfelachtig. Pogingen tot interventie op de boerderij of tijdens het slachtproces hebben daarom waarschijnlijk meer kans van slagen dan pogingen tot interventie bij de consument.

Geconcludeerd kan worden dat het uitvoeren van deze risicoschatting een nuttige en leerzame ervaring is geweest. De MPRM methodiek lijkt goed bruikbaar. QMRA 'van boerderij tot volksgezondheid' zal zich in de toekomst ten eerste moeten richten op een bruikbare 'statement of purpose' en op een beschrijving van de procesketen, met daarbij een inventarisatie van de gegevensbehoefte vóórdát alle beschikbare data opgespoord worden. De mogelijke discrepantie tussen modelcomplexiteit en gegevensbeschikbaarheid dient zorgvuldig te worden overwogen. Het ontwikkelen van vaardigheden in het bevragen van deskundigen verdient de nodige aandacht. Het uitvoeren van QMRA is ingewikkeld en tijdrovend. Het vraagt om verregaande integratie tussen verschillende werkterreinen als microbiologie, modellering en risicomanagement.

Summary

Quantitative microbiological risk assessment (QMRA) is increasingly used as a tool to evaluate food related health risks. Recently, the Modular Process Risk Model (MPRM) was proposed as a methodology to set up a 'farm to fork' risk assessment model, which quantitatively describes the transmission of a microbiological hazard through the food pathway. This not only allows an assessment of current health risks, but also offers the opportunity to compare the effects of risk management interventions proposed to reduce the risks.

QMRA is a relatively new and complex area of research. The objective of the study presented in this report was to further develop the methodology for conducting a QMRA 'from farm to fork and beyond', to discover potential pitfalls and to explore the available data and their suitability for QMRA. It integrates 'farm to fork' exposure assessment modelling with microbiological dose response modelling. The hazard considered is Shiga toxin producing *Escherichia coli* O157 (STEC O157), a notorious pathogen. Related illnesses range from mild gastro enteritis, haemorrhagic colitis, haemolytic uremic syndrome in young children, to death. Although no large-scale outbreaks of STEC O157 have occurred in the Netherlands so far, several major outbreaks have occurred abroad. Cattle is generally considered as the most important reservoir of STEC O157. Therefore, a beef product was chosen as the food product for which the risk is evaluated. Based on consumption frequency, potential risk and relative simplicity of processing, the product choice was 'steak tartare patties', a lean ground beef product, typically eaten raw or partially raw. To limit the complexity of the assessment, only the Dutch population and only data on Dutch animals and slaughterhouses were considered in the analysis.

In exposure assessment the food pathway 'from farm to fork' is described and modelled using the MPRM methodology. This implies that the food pathway is separated into basic processes. New models of the basic processes 'mixing' and 'partitioning' are developed that allow evaluation of the effect of non-random distribution of micro-organisms in the substrate. As slaughter practices may differ, three routes of exposure were compared, separating 'industrial' and 'traditional' ways of both slaughter and subsequent processing. Consumers were separated in three age classes, 1-4 years, 5-14 years and 15+, to fit with the effect modelling. Next, three preparation styles of the steak tartare patties (raw, medium and well done) were considered. (Dutch) data were collected on the prevalence and concentration of STEC O157 at the different stages in the food pathway: farm, slaughter, retail and consumer. This report offers an extensive overview of these data.

When the food pathway was modelled, it appeared that important information required to estimate the values of the model parameters was lacking. Therefore an expert elicitation workshop was organised to estimate the values of the remaining parameters for which no data were found. One of these parameters is for example the faecal contamination of carcasses, expressed in gram faeces per carcass. The model was implemented in an @Risk spreadsheet and Monte Carlo simulations were run. In these simulations, only variability was considered.

Uncertainty could not be quantified, due to its complex nature and the level of uncertainty in both the food pathway, the models and the data. As an alternative, a baseline model with (most likely) default values for the parameters is compared with alternative scenario's in which parameter values were modified for single parameters. This resulted in an evaluation of the effect of the uncertainty in single parameters on the end result of the risk assessment.

The exposure model predicts that about 0.3% of the raw steak tartare patties is contaminated with STEC O157. Of these contaminated patties, a large fraction (>60%) is contaminated with one colony forming unit (cfu) only. High contamination levels are rare, with for example only 7% of the contaminated raw steak tartare patties containing more than 10 cfu. In a microbiological survey it was found that one of 82 raw steak tartare patties (1.2%) was positive for STEC O157. Knowing that the probability of detection of single cfu's in such a survey is small, this suggests that the model prediction is an underestimation of the actual level of contamination of steak tartare patties.

The 'farm to fork' exposure model was linked to a dose response model of STEC O157, based on data of an outbreak in an elementary school in Japan in 1996. This outbreak is unique as it supplies accurate human data on dose and the number of people exposed and infected. The exponential and the hypergeometric model fitted to these data indicate that STEC O157 is highly infective. With the exponential model, the probability of illness per single cell is estimated at about 0.5%

Next, the number of STEC O157 infections by steak tartare consumption per year in the Netherlands was predicted with the baseline model at 2300, and the number of cases of gastro enteritis at about 1300. The latter equals an incidence rate of 8 per 100.000 person years. This result can be compared with an independent estimate of the total incidence of STEC O157 associated gastro enteritis in the Netherlands based on epidemiological data: 2000 cases or 13 per 100.000 person years. Apparently a large fraction of the cases is a consequence of steak tartare consumption. However, the uncertainty in these estimates is large. As many more routes of exposure to STEC O157 are known, the large attributable fraction of steak tartare consumption (that is the high contribution to the total incidence), seems an overestimation. In contrast, the contamination level in raw tartare seems to be underestimated by the model.

These qualitative conclusions hold when the uncertainty in the different parameter estimates is taken into account. The high incidence of illness appears to be particularly related to the dose-response model used. Other dose-response models predict considerably lower incidences of illness. However, all of these are based on proxies for the host or the pathogen. Nevertheless it is possible that the specific Japanese outbreak data are not representative for the general exposure to STEC O157 in steak tartare consumed by Dutch people, for example because a particularly virulent strain was involved.

The risk estimate is highly uncertain, but nonetheless the risk model can be used to investigate the impact of uncertainties in the model input on the model output, and to evaluate intervention at different stages along the food pathway. Analysis of alternative scenario's shows that the uncertainty in prevalence and concentration of STEC O157 at farm level may have a large effect on the final model estimates. The same holds for uncertainty about growth and inactivation of STEC O157 on the carcass. In contrast, the effect of growth of STEC

O157 during retail and domestic storage is negligible and the effect of advocating the consumption of 'well done' steak tartare patties is questionable. This suggests that intervention at farm level or at slaughter is more likely to be effective as a strategy to reduce STEC O157 associated risks than intervention at the consumer level.

As a conclusion, the risk assessment has been a valuable experience. The MPRM was a useful approach. In the future, the focus of QMRA 'from farm to fork and beyond' should first be on a manageable statement of purpose and a description of the food pathway making an inventory of data needs, before surveying the available data. The potential discrepancy between model complexity and data availability should be carefully considered. Also, developing expertise in expert elicitation is recommended. Finally, conducting QMRA is complex and time consuming. It needs an integrative approach, with microbiologists, modellers and managers involved.

1. Introduction

1.1 A Risk Assessment of pathogenic *E. coli*

Quantitative microbiological risk assessment (QMRA) modelling is increasingly used as a tool to evaluate food related health risks. Recently, an increasing number of papers have been published that describe QMRA studies for a range of micro-organisms and food products (Whiting and Buchanan 1997, Cassin et al. 1998, Marks et al. 1998, Coleman et al. 1998, Bemrah et al. 1998). So far, different approaches have been used, and several protocols and guidelines have been proposed (e.g. CODEX Alimentarius Commission 1998, ILSI 2000). Recently, we developed the Modular Process Risk Model (MPRM) methodology to structure exposure assessment modelling from 'farm to fork', and applied this to a QMRA of *Bacillus cereus* in a vegetable product (Nauta 2001b).

Risk assessment can be divided in four steps: hazard identification, hazard characterisation (including dose response assessment), exposure assessment and risk characterisation (e.g. CODEX Alimentarius Commission 1998). At the Microbiological Laboratory for Health Protection (MGB) within RIVM, expertise has been developed on these different steps. We now aim to integrate this expertise as described previously (Havelaar et al. 2000), and conduct a specific risk assessment 'from farm to fork and beyond', that is up to an evaluation of the health burden on population scale. To specify a risk assessment, one needs to identify a pathogen, a product, a food pathway and a population (see Nauta 2001b). For this study, we decided to study pathogenic *Escherichia coli* in a beef product consumed by the Dutch population.

An important reason to conduct a QMRA for pathogenic *E. coli* was its potential impact on public health. Outbreaks associated with pathogenic *Escherichia coli* have frequently been reported in the USA (69 outbreaks in the period 1982-1994) and other countries. As yet, no large outbreak has occurred in the Netherlands, but such an outbreak cannot be excluded for the future (Heuvelink 2000b).

The most important animal reservoir for Shiga toxin-producing *E. coli* O157 (STEC O157), the serotype receiving most attention, is cattle, although STEC can be found in the faecal flora of a wide variety of animals. Animals carrying STEC O157 are usually asymptomatic. STEC O157 can persist in manure, water troughs, and other places on cattle farms. Transmission to humans occurs via food of animal origin (mainly undercooked ground beef and raw milk), fruit and vegetables, water, person-to person and animal-to-person contact and occupational exposure. Ground beef is the vehicle responsible for the largest portion of foodborne STEC O157 outbreaks in the USA, including the large multistate outbreak in 1993 (Armstrong et al. 1996). This outbreak affected over 700 persons and caused the death of four children (Bell et al. 1994). Once infected with enterohaemorrhagic *E. coli*, people may develop gastro enteritis, including severe, bloody haemolytic colitis. Young children can

develop HUS (Haemolytic Uraemic Syndrome), a severe renal disease that may lead to renal failure and even to death (Heuvelink 2000c, Havelaar et al. 2001).

Another reason for selecting STEC O157 was the availability of recent data from research on this pathogen in the Netherlands (Heuvelink 2000b). Finally, this risk assessment on STEC O157 was facilitated by the experience of other risk assessments on this pathogen, performed in North America (Cassin et al. 1998, Marks et al. 1998, Coleman et al. 1998).

The main objective of the study described in this report was to further develop experience in conducting a risk assessment ‘from farm to fork and beyond’, and to discover potential pitfalls. This means that the risk assessment described here was not primarily aimed at assessing health risks or to be used as a tool for risk management.

1.2 Report outline and guide

This report describes several aspects of risk assessment and gives quite some details that reflect our experiences. Not all these details will be of interest for all readers. The outline below may therefore be used to select specific topics of interest. In general, each chapter can be read separately without studying the other chapters in detail.

In chapter 2 the statement of purpose is defined. It describes the process in which the choices were made that lead to the formulation of the statement of purpose, which includes definitions of pathogen, product, food pathway and population of concern. The statement of purpose is the important first step of any risk assessment (CODEX Alimentarius Commission 1998). It serves to clarify exactly what the risk assessment is about, and what it is aiming at. Because this was a feasibility study, choices were made after consultation with experts rather than risk managers as proposed by Codex.

Chapter 3 describes the exposure assessment. First, the food pathway for steak tartare production in the Netherlands is explained. Next, it gives an overview of available data on prevalence and concentration of STEC O157 at several stages along the food pathway, and data on consumption and food handling. Finally, the risk model is defined, using the MPRM methodology. The food pathway is modelled by splitting it up in a series of consecutive basic process models. Model parameter values are estimated on the basis of available data and expert opinion. The exposure assessment model is implemented in a spreadsheet program.

Chapter 4 deals with the dose response model for STEC O157, developed especially for this study on the basis of a Japanese elementary school outbreak (Shinagawa 1997). The data are fitted with both an exponential and a hypergeometric model. The resulting dose response models are compared with other dose response models for STEC O157 (Haas et al. 2000, Powell et al. 2000).

The risk characterisation, which integrates the exposure model with the dose-response model is given in chapter 5. It shows the results of the risk model, in terms of estimates for the prevalence and concentration of STEC O157 raw steak tartare patties, and the incidence of STEC O157 associated illness in the Dutch population. Next to the baseline model,

alternative scenarios are analysed. The model results are compared with independent data for the purpose of validation.

Chapter 6 deals with quantifying and modelling the effect of an information campaign and describes previous experiences with information campaigns related to public health. Such a campaign is a possible intervention measure to reduce the incidence of STEC O157 related diseases.

Finally, in chapter 7 the risk assessment exercise is reviewed. The relevance of the risk model results for the public health risk of STEC O157 in the Netherlands and the modelling approach are discussed. Lessons on risk assessment modelling are summarised.

2. Defining microbial risk assessment

2.1 Generic statement of purpose

Risk assessment should start with defining a clear statement of purpose (CODEX Alimentarius Commission 1998), because it is important to have a clear goal of the project for all co-workers. At the start of the project, ample time was spent on generating a clear-cut statement of purpose. Danger of a clear-cut statement of purpose is that it becomes unrelentingly clear if a project in the end does not comply with this statement of purpose. This was partly the case for this project as stated in section 2.7.

The statement of purpose of the risk assessment was originally formulated in a more or less generic sense as follows:

- 1) Assess the exposure distribution for a pre-defined category of Dutch consumers to the pathogen in a specific product, produced in a specific pre-defined process;
- 2) Assess the health burden that results from the exposure assessed above;
- 3) Assess the effect of one or two risk mitigation strategies proposed by selected experts (“risk managers”) at the start of the project.

In sections 2.2 to 2.6 below, restrictions to the risk assessment will be obtained, resulting in section 2.7 in the clear-cut statement of purpose to be used in this study.

2.2 General

In general, and in this case also, time is limited to execute a microbial risk assessment. Therefore, it is not realistic to execute ‘a microbial risk assessment for *Escherichia coli*’. Moreover, this is a The baseline demarcation for this project was made beforehand. The idea was to perform a microbial risk assessment for *E. coli* with ground beef as transmission route. However, more detailed demarcations were necessary to obtain a realistic project size.

These are:

- product definition: exactly which ground beef product is to be considered;
- species/serotype definition: exactly which type or set of types are to be considered;
- interventions: two interventions was considered a reasonable number.

The output of the risk assessment, the health burden (point 2 of the statement of purpose), was not specified further.

Two sessions with experts were undertaken to make the choices.

- session 1: with food microbiologists from the Inspectorate for Health protection and Veterinary Public Health;
- session 2: with food and veterinary microbiologists from the National Institute of Public Health and the Environment.

Mathematical modellers from the National Institute of Public Health and the Environment made the definitive decisions for interventions in a subsequent session. A definitive decision on product choice was made on the basis of a limited investigation of consumption amounts.

2.3 Product choice

The risk assessment is restricted to products that are produced from meat of Dutch slaughterhouses whether from Dutch or foreign origin. So imported products but not imported live animals are excluded. We did not limit the risk assessment to meat from Dutch cattle only, as it proved to be impossible to discriminate between Dutch and foreign cattle after slaughter. This means we will have to try to collect data on prevalence and concentration of STEC O157 in foreign live cattle too.

2.3.1 Sessions

Criteria

Two criteria were passed to the session experts:

- Preference is given to that product for which processing in the butcher's shop and preparation in the household is the simplest;
- Preference is given to that product for which the estimated public health risk is the largest.

It is realised that these criteria are contradictory: a simpler processing scheme generally gives a lower public health risk, because there are less moments of possible (cross-) contamination.

Session 1

Hamburger, tartare, minced meat and filet americain were considered. Tartare is minced beef with a fat content of less than 10 % and filet americain is tartare mixed with a mayonnaise-based sauce (80 to 20 %) (Heuvelink et al. 1999a, Heuvelink 2000f). Minced meat was discarded as this can be prepared in the household in many ways. Filet americain was discarded as there is much variation between butchers in the way it is prepared. Also it is usually not consumed by children, whereas children must be included because of the disease HUS.

This leaves the choice between hamburger and tartare. Three public health arguments plead for tartare:

- 1 Tartare is thicker than a hamburger, therefore the risk of insufficient heating of the centre is larger;
- 2 People tend to accept a partially raw tartare but do not accept a partially raw hamburger;
- 3 Tartare is sometimes consumed raw: a tartare roll in e.g. snack bars.

One simplicity argument pleads for tartare:

- 4 The role of cross contamination is relatively limited as it is not used for barbecue.

One public health argument pleads for hamburger:

1 Hamburgers are used for barbecue which is a public health risk due to cross contamination between raw and prepared hamburgers, and inadequate cooking.

More arguments plead for tartare and therefore this product is chosen in first instance. However, it was thought that a definitive choice should also depend on consumed amounts.

Session 2

Products were briefly discussed. Filet americain is thought to be not very risky as it is made directly from a piece of steak. One expert chose for hamburger, because of consumed amounts. The other expert could not choose between hamburger and tartare.

2.3.2 Consumption amounts of tartare and hamburger

Introduction

The first selection of minced products as described above left two minced products: tartare and hamburger, with some preference for tartare. However, it was thought reasonable that eventually a choice for hamburger would be made if the amount consumed would be much higher, say a factor 10 or more, than that of tartare. A higher amount consumed results in a higher public health risk and a subjective limit of a factor 10 was set to revert the product choice from tartare to hamburger. Setting a more objective limit would require a time-consuming investigation.

Data

Two data sources were used to estimate the amount of consumption, both based on field research by GfK PanelServices Benelux:

- Data obtained from the Product Boards for livestock, meat and eggs (PVE);
- A report from TNO Nutrition and Food Research Institute in which results of the third Dutch nutrition surveillance are presented (Kistemaker et al. 1998).

The data from PVE are expressed in kg of a product per year. Assumptions used for the calculations below:

- The percentage of household of total purchase for hamburgers does not deviate much from that for the category 'ready to cook/snacks in general', which includes hamburgers and equals 53 %;
- The household category 'other minced meat' is for the main part tartare;
- The percentage of household of total purchase for tartare does not deviate much from that for the category 'minced meat' in general, which includes tartare and equals 86 %.

From Kistemaker et al. (1998) the data were used that refer to a research panel of 6250 people including households with heads 75 years or older of age (Table 1 in that report). Food consumption data were registered by these people for two consecutive days. For each product, the mean consumption per day (g) of users was multiplied by the number of users and thereafter divided by the total number of people (6250) to arrive at the mean

consumption per day for all people. For comparison with PVE data, this number was multiplied by 15×10^6 people and 365 days and divided by 1000 to arrive at the annual Dutch consumption in kg. Also, the mean number of products consumed per day for all people was estimated, assuming that nobody consumed a product on both days.

Results for hamburger

PVE data: household purchase is 11×10^6 kg. Total purchase is then estimated at 20.8×10^6 kg.

TNO data: addition of two products, 'hamburger raw' (code 1435) and 'hamburger prepared' (code 1569), gives a value of 3.03 g pppd (per person per day). The annual Dutch consumption is then estimated at 16.4×10^6 kg, agreeing reasonably with the PVE figure. The mean number of hamburgers consumed per day for all people was estimated at 0.037.

Results for Tartare

PVE data: household purchase is 2.4×10^6 kg. Total purchase is then estimated at 2.8×10^6 kg.

TNO data: addition of two products, 'beef tartare raw' (code 1415) and 'beef tartare prepared' (code 1550), gives a value of 1.70 g pppd. The annual Dutch consumption is then estimated at 9.3×10^6 kg, more than a factor 3 higher than the PVE figure. The mean number of beef tartare consumed per day for all people was estimated at 0.018.

Comparison

The ratio of hamburger to tartare is:

- according to PVE data (weight): 7.4 to 1;
- according to TNO data (weight): 1.8 to 1;
- according to TNO data (pieces): 2.1 to 1.

None of these figures are greater than 10, the limit set above to choose for hamburger as product. Moreover the PVE data are less reliable as they are not as product specific as the TNO data. Therefore tartare is chosen as product for the microbial risk assessment.

2.4 Species/serotype definition

Three criteria were passed to the experts:

- The type should constitute a public health risk;
- Measurements should be available;
- The choice should be practicable.

STEC O157, which is synonymous to VTEC O157, was chosen as the serotype for the risk assessment (STEC = Shiga toxin-producing *E. coli*; VTEC = verotoxigenic *E. coli*). The choice was made in session 1 and not rejected in session 2. This choice was data-driven: STEC O157 is the only serotype for which measurements are available. Based on medical data, the importance of non-O157 STECs is currently limited (Armstrong et al. 1996). An

exact definition of STEC O157 is: *E. coli* O157-strains that are VT1 and/or VT2 positive. The *eae* status is not part of the definition, however STEC O157 strains are usually *eae* positive.

2.5 Choice of interventions

It was made clear to the experts that choice of interventions is important as the model is to be made more detailed in the parts related to the proposed interventions. The choice was to be limited to two interventions at different positions in the chain farm – slaughterhouse (carcass processing) – butcher's shop/supermarket (meat (product) processing) – household/restaurant. It must be stressed that in session 1 and 2 below the opinion of the consulted experts is reflected.

Session 1

Two links in the chain were considered less suitable for interventions, namely farm and butcher's shop. The farm is less suitable as contamination routes are currently unclear and as the farm sector is relatively difficult to direct. Interventions at the butcher's shop seem less relevant. Cross-contamination is the most important danger here. Points of interest are:

- Separate meat products ready for consumption and meat products to be heated prior to consumption;
- Cleaning the meat mincer;
- Good storage conditions (temperature);
- End product treatment (radiation).

The first link to be considered is the final stage in the farm combined with the slaughter house. Less suitable interventions for this stage are:

- 'Flaming' risky parts of the carcass. This influences meat quality;
- Starving the cows a short time period prior to slaughter, resulting in less filled intestines.

A better intervention would be to clean the cows before they enter the slaughter house. This includes two partial interventions:

- Cleaning at the farm: removing visible faeces with brush and water;
- Cleaning the transport truck.

The best intervention is to improve hygiene of slaughter. The two most important partial interventions are improvement of hygiene with respect to:

- Dehiding, e.g. avoiding contact between hide and carcass meat;
- Removal of intestines, e.g. by a peg on or a bag below the rectum.

The second link to be considered is household/restaurant. Focus will be on households rather than restaurants, because most food poisonings occur in households and because outbreaks,

which are usually related to restaurants, seldom occur in the Netherlands. Good interventions would be:

- Putting labels with a text on packaged meat, probably also resulting in a discussion which has positive effects in itself;
- Giving education on secondary schools.

The best intervention according to the experts is starting an information campaign. The message should be: all raw meat is potentially contaminated with pathogens, take care. Attention should be given to heating and cross contamination.

Session 2

Interventions and best interventions were selected for the four links and thereafter the two overall best interventions were selected.

Good interventions for the farm are:

- Keeping it closed, that is, not allowing animals from outside other than via quarantine;
- Selecting feed that reduces infection with STEC O157 (there is no scientific agreement on the properties of such feed);
- Using GMP-produced feed;
- Separating age groups of cows, as young animals are more sensitive to infection.

The best intervention for the farm is hygiene interventions, such as removing feed rests and cleaning drinking water bowls.

Interventions for the slaughter house are:

- Surveillance for STEC O157 at cattle farms, applying strict hygiene interventions for slaughter if positive;
- Decontamination of raw meat at the end of the slaughter line;
- Hygiene interventions;
 - Spray cleaning of cattle before slaughter (removing faeces)
 - ‘Shaving’ the animals, possibly after killing (removing faeces)
 - More supervision on currently prescribed interventions
 - Not starving the cows before slaughter, which reduces the risk of infection

The best intervention is also a hygiene intervention, namely logistic slaughter. Logistic slaughter means that on a day, the not infected animals are slaughtered before the infected animals. An additional effect is that flows of meat go separately to the butcher’s shop.

Interventions for the butcher’s shop are:

- Preventing growth by controlling temperature and cleaning;
- Complete separation of raw and prepared products;
- Execute internal surveillance by taking microbiological samples.

The best intervention is prevention of cross contamination, meaning contamination of meat by employees or by microbial populations that are resident in the butcher’s shop.

The best intervention for households is hygiene education at primary and secondary schools. Interventions for restaurants are hygiene interventions, such as cleaning and separate lines. The best intervention is increasing the number of samples taken at Critical Control Points. Preference is given to interventions at the beginning of the food chain. A greater risk reduction is realised by reducing the initial contamination level than by (hygiene) interventions in links later in the food chain. Furthermore, interventions for the households will only be effective in the long term. Therefore the following two interventions are chosen:

- Hygiene interventions at the farm;
- Logistic slaughter at the slaughter house.

Subsequent session

From the two previous sessions, four interventions were selected:

- Hygiene interventions at the farm (feed residues; drinking water) (session 2);
- Logistic slaughter (session 2);
- Improving hygiene at slaughter (dehiding; removal of intestines) (session 1);
- Execute an information campaign (session 1).

The only intervention that seems to be relatively simple to implement, is ‘logistic slaughter’. However, there are problems: intermittent shedding of STEC O157, the absence at present of a fast test kit for screening of animals at slaughter, the necessity to test the main part of the animals, but these problems seem relatively minor. Furthermore, some work already has been done on this subject. Therefore, this intervention is selected. As it is preferred to spread interventions along the food chain, the intervention ‘improving hygiene at slaughter’ is discarded. See (Gannon 1999) for a review on control of *E. coli* O157 at slaughter and (Roessink and Bosboom 1999) and (Heuvelink et al. 2001) for information on the related zero-tolerance program for slaughter houses in The Netherlands.

This leaves ‘hygiene interventions at the farm’ and ‘information campaign’. ‘Hygiene interventions at the farm’ is difficult because of unclear contamination routes, difficulties in directing and complex modelling. See (Hancock et al. 1998) for a review of on-farm ecology of *E. coli* O157 and possible control strategies. ‘Information campaign’ suffers from the fact that the effect is doubtful and that it is difficult from a political point of view. It was decided to start with the intervention ‘information campaign’, with the argument that then one intervention from each session will be investigated. If it would prove to be very difficult to implement this intervention, then ‘hygiene interventions at the farm’ would be chosen as intervention to be investigated.

2.6 Consumer definition

Consumption data were obtained from the Dutch nutrition surveillance (Kistemaker et al. 1998). This implies that the sample population definition used in this surveillance sets the exact consumer definition used in the present study also. This definition is: persons that live

in households in The Netherlands and do not live in institutions, have sufficient knowledge of the Dutch language, and are 1 year or older. Dutch nationality is no prerequisite.

2.7 Statement of purpose

Based on section 2.1 to 2.6 above, the statement of purpose can be formulated as:

1. Assess the exposure distribution to STEC O157 in tartare for consumers in The Netherlands;
2. Assess the health burden that results from the exposure assessed above;
3. Assess the effect of the interventions ‘logistic slaughter’ and ‘information campaign’.

As will be clear from the remainder of this report, part 3 of the statement of purpose was not realised, mainly because of project time limitations. As for information campaigns, a number of institutes were visited with primary aim to obtain quantitative information on the effects of a campaign. The information obtained from these institutes is presented in chapter 6. It is not readily implementable in the model, but gives insight in the state of the art of knowledge on information campaign effects and might also be useful in future risk assessment projects.

3. Exposure assessment

3.1 The Food Pathway

3.1.1 Introduction

In Section 3.1 a description of the flow of meat and the accompanying pathogen and also starting points for modelling that were defined at the start of the project are given. At the same time, this section gives insight into developments that took place during the execution of the project. First, a simple scheme was developed using mainly knowledge of the authors at that moment (Section 3.1.2). Second, an improved scheme was developed using mainly knowledge from the Inspectorate for Health Protection and Veterinary Public Health (Section 3.1.3). Finally, this scheme is to be compared with the scheme that is eventually used in risk assessment modelling (Figure 3-6 and 3-7). Also, the initially defined starting points for modelling (Section 3.1.4) are to be compared with the final risk model (Section 3.3).

3.1.2 A simple scheme

A meat product can be produced in many ways. There can be variation in the origin and type of the animals, the slaughtering process, meat processing and the way of preparation. A 'generic' process will be modelled.

In Figure 3-1 a simple scheme is presented that was used as a starting point for this risk assessment study. The flow of events consists of two parts:

- Exposure modelling, from cattle in the farms to a prepared product with a dose just before consumption;
- Effect modelling, from a prepared product with a dose just before consumption to public health burden expressed in Disability Adjusted Life Years (DALY's).

In a number of cases, the flow was divided in two or more subflows. This was a preliminary subdivision, to be maintained only if the subflows would prove to have different characteristics. The chain starts with the farm, in which, first, Dutch and foreign cattle and, second, veal calves, dairy cattle and veal cattle are distinguished, leading to six categories. After transport the cattle is slaughtered in the slaughterhouse. Again after transport or otherwise the carcasses are further processed into a product in either an industrial or a traditional setting. It was assumed that the two flows remain separate, the 'industrial' meat ending up in a supermarket after transport and the 'traditional' meat ending up in a butcher's shop. Then the flows were supposed to intermingle and after transport the meat ends up in either a restaurant or in a private household. There it is prepared for consumption. Along with

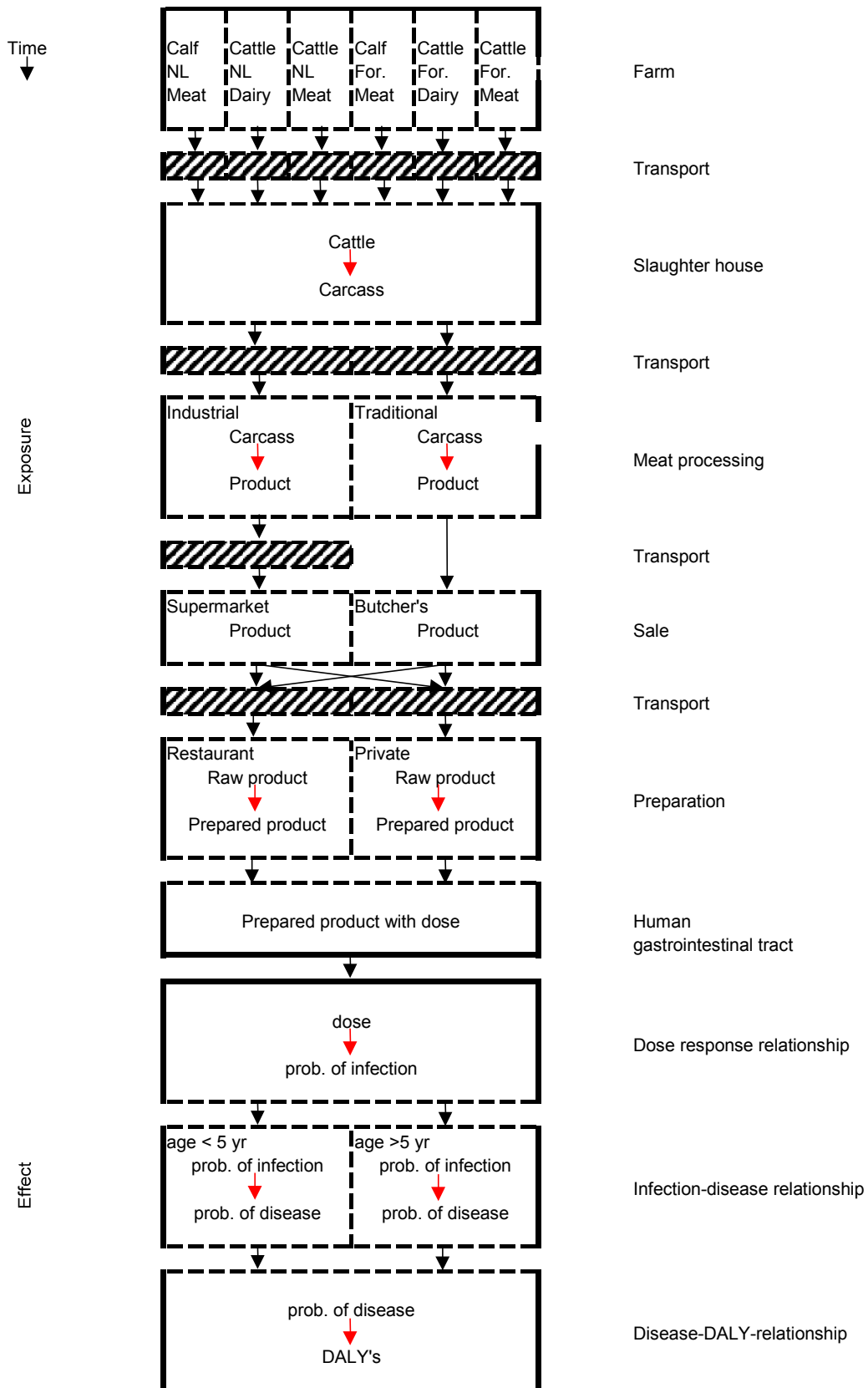


Figure 3-1 Simple scheme for farm to public health process flow. NL = Dutch, For. = Foreign.

the consumed product a certain dose of micro-organisms is ingested. At this point in the food chain exposure modelling ends and effect modelling starts.

A dose response relationship results in the probability of infection. Starting from this probability, two flows to the probability of disease that are separated by an age limit of 5 years were distinguished in the simple scheme (Figure 3-1). This distinction is related to the disease of HUS. Finally, the probabilities of diseases in combination with population size can be used to estimate the public health burden expressed in DALYs.

3.1.3 Improved scheme

The general Scheme (Figure 3-1) was made more specific for the present risk assessment, mainly based on information obtained from the Inspectorate for Health Protection and Veterinary Public Health. This resulted in Figure 3-2. In this scheme, three types of butchers are distinguished:

- the “cattle” butcher that slaughters cattle himself;
- the “carcass” butcher that receives half carcasses from the industrial slaughter house and does the meat cutting himself; and
- the “meat” butcher that receives the fresh meat and does very little processing himself.

Meat production firms deliver products that have no fresh meat features any more due to e.g. heating, addition of herbs, marinating, etc. Wholesale firms only function as middleman. The distribution centres have a buffer and distribution function. In order to avoid a ‘forest of arrows’ in Figure 3-2, meat flow coming from the industrial deboning plant always goes via a distribution centre, but this should be interpreted as optional, meat might go via a distribution centre, and it might not. Restaurant chains are on the one hand companies that have a separate processing chain of their own, and on the other hand companies that do not have this.

The origin of cattle meat is twofold. On the one hand, there is the male part: fattening firms that fatten male cattle to a lower age (veal calves) or higher age (veal cattle). On the other hand, there is the female part: cows from dairy farms. The bulk of tartare will probably be produced from meat of dairy cattle and veal cattle, as the white meat from veal calves will probably seldom be used for tartare.

For public health now three age categories are distinguished (as opposed to the simple scheme of Figure 3-1 which uses two): 1- 4 years, 5-14 years, and older.

An important aspect for the interpretation of a risk assessment is the extent of import and export. For the cattle sector in the Netherlands in 1998, meat flows are depicted in Figure 3-3. The data were obtained from the Product Boards for Livestock, Meat and Eggs (PVE 2001). The Dutch consumption of cattle meat (289×10^6 kg) is much larger than calve meat, which amounts to 20×10^6 kg. The calve sector thus constitutes only 6 % of the total (cattle + calve) sector, which makes it relatively unimportant for risk assessment of tartare if it is assumed that there is no strong preference for calve meat for tartare production. From Figure 3-3 the following observations can be made:

- Only a small part (5%) of Dutch production is exported alive;

- Also a small part (9%) of slaughter is living import;
- An important part (67%) of slaughter is exported;
- An important part (61%) of Dutch consumption consists of imported meat (products).

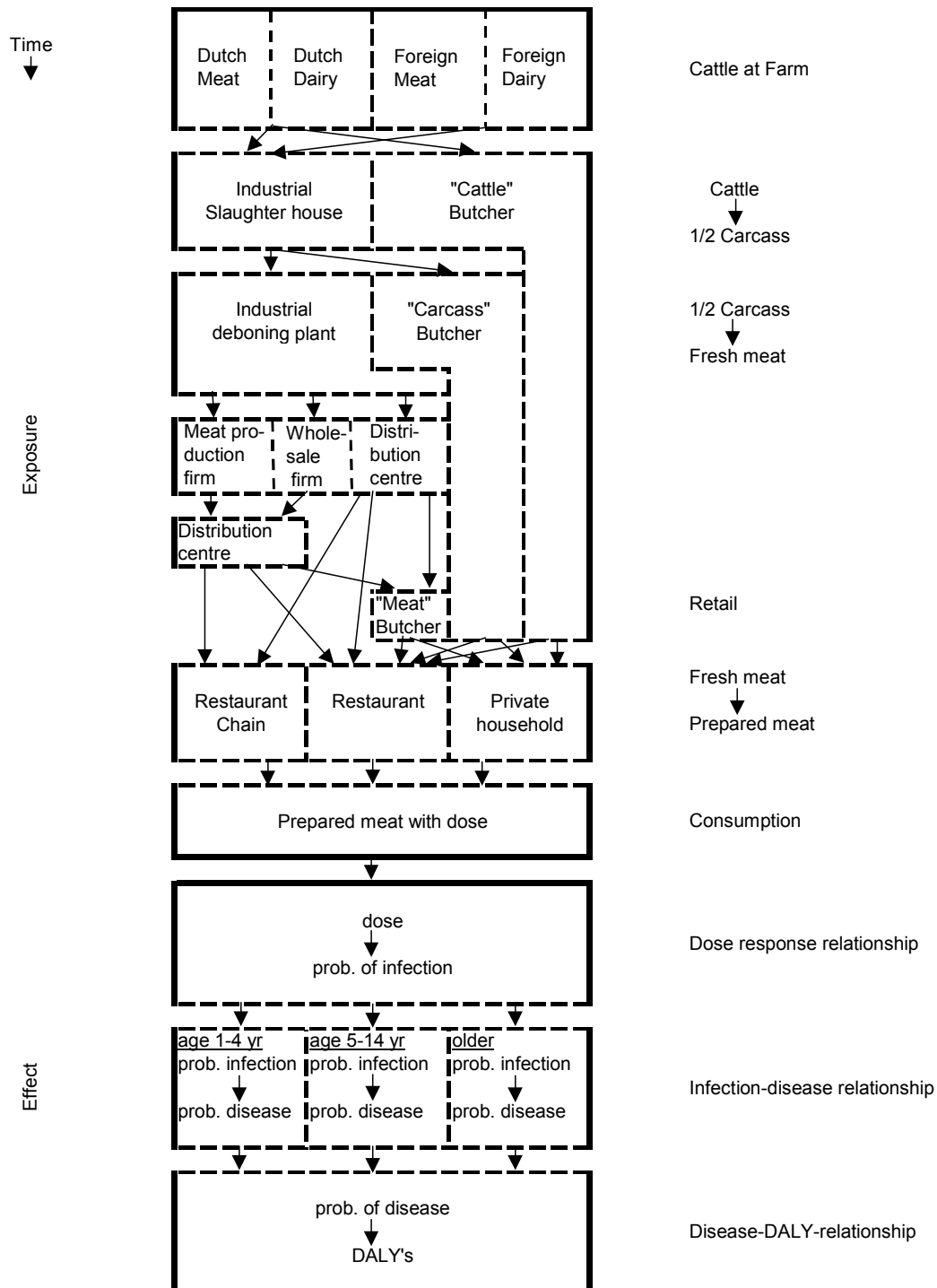


Figure 3-2 Improved scheme for farm to public health process flow

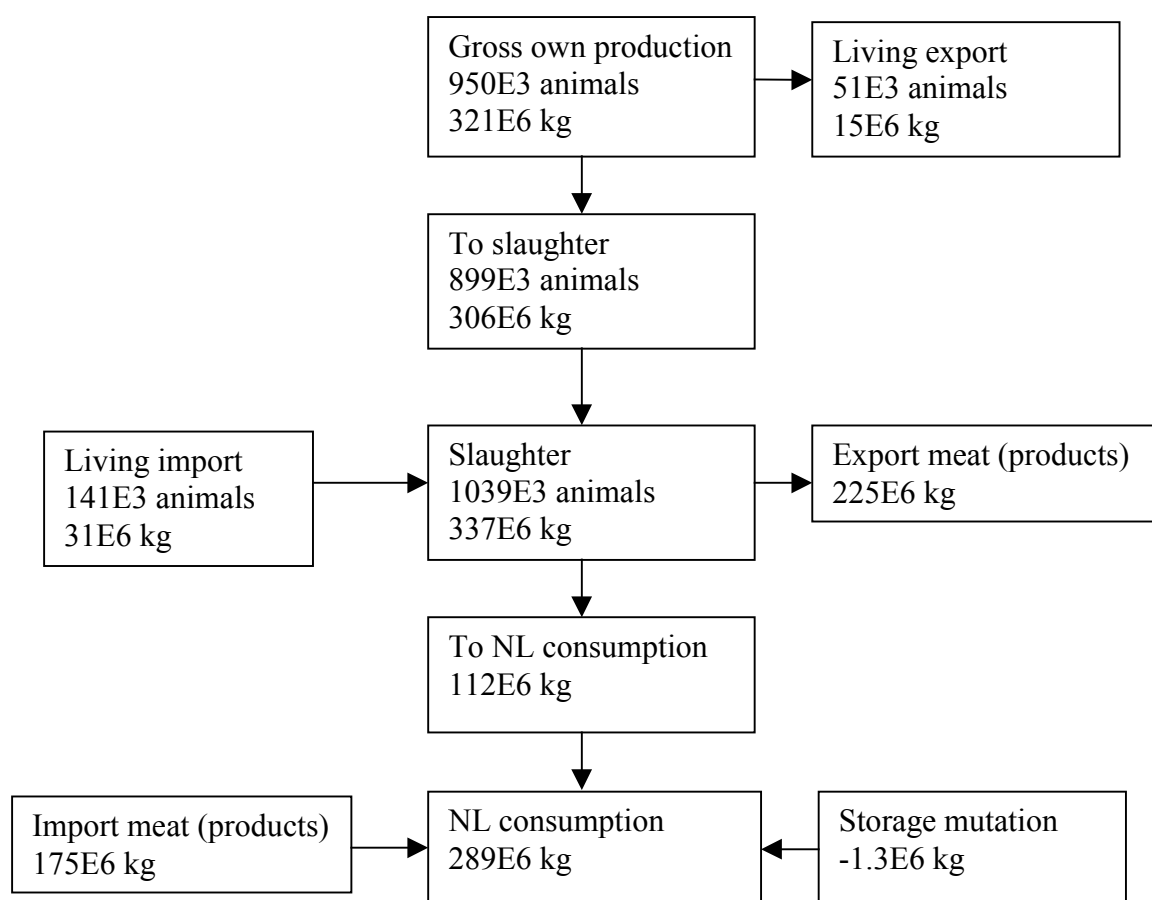


Figure 3-3 Meat flow scheme for the cattle sector in the Netherlands in 1998.

It must be stressed that these are numbers for cattle meat as a whole, therefore drawing conclusions for tartare must be done with care. For risk assessment, import and export are important if STEC O157 prevalence and/or concentrations differ much between The Netherlands and foreign countries. One can choose for a risk assessment for tartare produced from:

- Cattle from Dutch farms;
- Cattle slaughtered in the Netherlands;
- All sources (Dutch cattle, living import and import of meat (products)).

For the last two options in principle foreign STEC O157 data are required. In the present risk assessment we chose (see the beginning of section 2.3) to restrict the risk assessment to tartare produced from meat originating from Dutch slaughterhouses, which corresponds to the second option.

3.2 Data

3.2.1 Prevalence

Dutch prevalence data are presented in full in Appendix 2.1 - 2.4. Below, these data are summarised and interpreted. In the tables, the references are preceded by a code which refers to the exact section of the Appendix where extensive information on the reference can be found. The data are subdivided into three links: farm, slaughterhouse and retail. First, three tables are given containing all relevant references. The farm references are presented in Table 3-1. These references are subdivided into on the one hand, references on dairy cattle, veal calves and veal bulls and on the other hand, references on the prevalence at farm and animal level, the time dynamics on farm and animal level, the length of the individual excretion period and the relationship between age and excretion. Table 3-2 presents the slaughterhouse references. The references are again subdivided into dairy cattle, veal calves and veal bulls. Also, analogous to the previous table, a subdivision is made into the prevalence at farm and animal level and time dynamics at animal and farm level. Table 3-3 presents the retail references. For these, a subdivision is made into references that are more relevant or less relevant. Most useful are references with data on tartare and STEC O157. Less useful are references with data for which either the product or the serotype definition, or both, are too broad.

Table 3-4 - 3-6 give a summary of the data. Table 3-4 describes farm data. Data on animal and farm level are again given separately. For animal level, it is indicated whether samples were taken at farms considered previously 'negative' or 'positive'. An attempt is made to give an impression of variation with time or farm. At animal level, these two sources of variation usually are mixed and thus difficult to separate. The number of sampling events, the number of samples per sampling event and the number of visited farms is presented. A higher number of sampling events and of farms gives a better impression of the variation related to time and farm, respectively. Finally, it is indicated whether the investigation was performed for a minimum of one year or not.

At farm level, data are available for dairy cattle and veal calves and are lacking for veal bulls. The prevalence at farm level varies per quarter of a year from 0 - 19 % and 0 - 25 % for dairy cattle and veal calves, respectively. At animal level, data are available for dairy cattle and veal bulls and are lacking for veal calves. For dairy cattle, prevalence at animal level varies from 0 - 9 % and 0 - 61 % for farms found previously negative and positive, respectively. For veal bulls, prevalence at animal level varies from 10 - 36 % for farms found previously positive.

Table 3-5 describes slaughterhouse data. Data on herd and animal level are given separately. Sampling details given are: the number of sampled animals, herds and slaughterhouses, the length of the sampling period and the frequency within this period. Information on variation related to herds or slaughterhouses was not or limited available. Variation per sampling

period is indicated. At herd level, data are available for veal calves and veal bulls but not for dairy cattle. Prevalences at herd level are 0 % and 0 - 33 % for veal calves and veal bulls, respectively. At animal level, data are available for all categories. Prevalences at animal level are 9 - 11 %, 0 - 1 % and 0 - 15 % for dairy cattle, veal calves and veal bulls, respectively.

Table 3-6 describes retail data. For these, no information is available on variation with location. Prevalence at product level was 2.6 and 0 % in 1996 and 1997, respectively, and no further details on sampling moment were available.

It must be noted - perhaps unnecessarily - that the measured prevalences depend on the sensitivity of the method and the way of processing of the samples (e.g. pooling).

Table 3-1 References on prevalence of STEC O157 in cattle at the farm, at farm and animal level. Be aware that prevalence at animal level is not from a random sample of farms.

Data type	Dairy cattle	Veal calves	Veal bulls
Prevalence farm level	2.1.F2 Reinders and Bijker 1999	2.1.F3 Heuvelink et al. 1999b	
	2.1.F3 Heuvelink et al. 1999b	2.1.F4 Van de Giessen 1998	
	2.1.F4 Van de Giessen 1998	2.1.F5 Van de Giessen 1999	
	2.1.F5 Van de Giessen 1999	2.1.F6 Van de Giessen 2000	
	2.1.F6 Van de Giessen 2000		
	2.1.F1 Heuvelink et al. 1998b Heuvelink 2000d (2x)		2.1.F2 Reinders and Bijker 1999
Time dynamics farm level	2.1.F2 Reinders and Bijker 1999 (2x)		
	2.1.F7 De Bodt 2000		
	2.1.F1 Heuvelink et al. 1998b Heuvelink 2000d (2x)	2.1.F3 Heuvelink et al. 1999b	2.1.F2 Reinders and Bijker 1999
	2.1.F2 Reinders and Bijker 1999	2.1.F4 Van de Giessen 1998	
	2.1.F3 Heuvelink et al. 1999b	2.1.F5 Van de Giessen 1999	
	2.1.F4 Van de Giessen 1998		
	2.1.F5 Van de Giessen 1999		
2.1.F7 De Bodt 2000			
Time dynamics animal level	2.1.F1 Heuvelink et al. 1998b Heuvelink 2000d		2.1.F2 Reinders and Bijker 1999
	2.1.F2 Reinders and Bijker 1999		
	2.1.F7 De Bodt 2000		
Length individual excretion period	2.1.F1 Heuvelink et al. 1998b Heuvelink 2000d		
	2.1.F2 Reinders and Bijker 1999		
	2.1.F7 De Bodt 2000		
Age and excretion	2.1.F1 Heuvelink et al. 1998b Heuvelink 2000d	2.1.F3 Heuvelink et al. 1999b	

Table 3-2. References on prevalence of STEC O157 in cattle at the slaughterhouse at farm and animal level. *: cattle originating from dairy farms.

Data type	Dairy cattle	Veal calves	Veal bulls	Veal bulls and/or dairy cattle
Prevalence farm level		2.2.S7 Reinders 2000	2.2.S6 Reinders and Bijker 1999	
Prevalence animal level	2.2.S3 Heuvelink et al. 1998a Heuvelink 2000g	2.2.S2 Heuvelink et al. 1996 (2x) 2.2.S3 Heuvelink et al. 1998a Heuvelink 2000g 2.2.S4 Reinders et al. 1997a 2.2.S5 Reinders et al. 1997b 2.2.S7 Reinders 2000	2.2.S6 Reinders and Bijker 1999	2.2.S1 De Boer et al. 1994 2.2.S2 Heuvelink et al. 1996 *
Time dynamics farm level		2.2.S7 Reinders 2000	2.2.S6 Reinders and Bijker 1999	
Time dynamics animal level		2.2.S7 Reinders 2000	2.2.S6 Reinders and Bijker 1999	

Table 3-3 References on prevalence of STEC O157 at retail.

Data type	Tartare & STEC O157	Product too broad	Serotype too broad	Product and Serotype too broad
Prevalence	2.3.R6 De Boer et al. 1997	2.3.R2 Van Heerwaarden and De Boer 1993	2.3.R3 Heuvelink et al. 1994	2.3.R1 De Boer et al. 1992
	2.3.R8 Heuvelink 2000a	2.3.R4 Heuvelink et al. 1996 Heuvelink 2000e		
		2.3.R5 De Boer et al. 1996		
		2.3.R7 Heuvelink et al. 1999a Heuvelink 2000f		

Table 3-4 Summary of literature data on prevalence of STEC O157 in cattle at the farm, at farm and animal level. '%': the prevalence. '-': and '+': farms found previously negative and positive, respectively. 'var': type of variation given in the column '%'. 'q.v.': quarterly variation. 'fsv': farm and seasonal variation. 'sv': seasonal variation. 'n/s': number of animals sampled per sampling event. 's': the number of sampling events, 'f': the number of farms. 'Part' and 'year': studies done for part of a year and at least a whole year, respectively. ^a: 2.1.F1 is two studies with overlapping data. ^b: E. coli O157.

Type	Prevalence	Study	%	var	n/s	s	f	period
Dairy cattle	Farm level	2.1.F2 Reinders and Bijker 1999	5.9	-	20	37	34	year
		2.1.F3 Heuvelink et al. 1999b	0-10.3	qv	1-60	82	82	part
		2.1.F4 Van de Giessen 1998	0-9.2	qv	1-60	267	267	year
		2.1.F5 Van de Giessen 1999	0-19.2	qv	1-60	161	161	year
		2.1.F6 Van de Giessen 2000 ^b	1.8	-	1-60	55	55	part
		2.1.F1 Heuvelink et al. 1998b Heuvelink 2000d ^a	-: 0-5.0 +: 1.9-22.4	f(s)v f(s)v	73-195 67-162	5	5	part
Veal calves	Farm level	2.1.F2 Reinders and Bijker 1999	-: 0-9.1 +: 0-61.0	(f)sv (f)sv	22-131 38-106	11	2	year
		2.1.F7 De Bodt 2000	+: 0-31	(f)sv	≈70-81	≈26	2	part
		2.1.F3 Heuvelink et al. 1999b	+: 0-30.3	sv	56-119	8	1	part
		2.1.F4 Van de Giessen 1998	0-12.5	q.v.	1-60	113	113	part
		2.1.F5 Van de Giessen 1999	0-20.0	q.v.	1-60	152	152	year
		2.1.F6 Van de Giessen 2000 ^b	7.1-25.0	q.v.	1-60	60	60	year
			3.1	-	1-60	32	32	part
Animal level	-	-	-	-	-	-	-	-
Veal bulls	Farm level	-	-	-	-	-	-	-
Animal level	-	-	+: 9.7-36.4	(fs)v	11-40	5	3	part

Table 3-5. Summary of literature data on prevalence of STEC O157 in cattle at the slaughterhouse at herd and animal level. '%' is the prevalence, possible variation is per sampling period, only 'veal bulls animal level' is per sampling period and slaughterhouse. 'n' is the number of sampled animals. 'h' is the number of herds. 'sl' is the number of slaughterhouses. 'Part' and 'year' refers to studies done for part of a year and at least a whole year, respectively. frequency is the frequency of sampling events.

Type	Prevalence	Study	%	n	h	sl	period	frequency
Dairy cattle	Herd level	-	-	-	-	-	-	-
	Animal level	2.2.S3 Heuvelink et al. 1998a	9.3, 11.1	540	-	5	2 x part	weekly
Veal calves		Heuvelink 2000g						
	Herd level	2.2.S7 Reinders 2000	0	≈840	28-56	1	year	monthly
	Animal level	2.2.S2 Heuvelink et al. 1996	0.9	365	-	1	part	weekly
		2.2.S3 Heuvelink et al. 1998a	0.5, 0.5	397	45	4	2 x part	weekly
		Heuvelink 2000g						
		2.2.S4 Reinders et al. 1997a /	0	273	-	1	part	weekly
		2.2.S5 Reinders et al. 1997b						
Veal bulls	Herd level	2.2.S7 Reinders 2000	0	≈840	28-56	1	year	monthly
	Animal level	2.2.S6 Reinders and Bijker 1999	0-33.3	285	53	1	3 x part	-
Veal bulls and dairy cattle	Herd level	2.2.S6 Reinders and Bijker 1999	0-15.0	329	-	2	4 x part	-
	Animal level	2.2.S1 De Boer et al. 1994	0	550	-	-	part	-

Table 3-6 Summary of (literature) data on prevalence of STEC O157 in tartare. '%' is the prevalence at product (tartare) level. 'p' is the number of tartares sampled. No information was available on the moment of sampling within the studies.

Product	Prevalence	Study	%	p	Period
Tartare	Product level	2.3.R6 De Boer et al. 1997	2.6	39	-
		2.3.R8 Heuvelink 2000a	0	43	-

Average prevalence values were calculated to exclude time dynamics. For this, only year-round data can be used if one wants to avoid making assumptions. The calculation for dairy cattle at the farm is shown below. Table 3-7 gives the usable data at farm level. The average prevalence at farm level can be calculated as $35/599 = 0.058$.

Table 3-7. STEC O157 farm level prevalence data of dairy cattle at the farm.

Study	No. of positive farms	No. of farms	Data from	Overall prevalence
2.1.F2 Reinders and Bijker 1999	2	34	Jan-Dec 1997	
2.1.F3 Heuvelink et al. 1999b	6	82	Apr-Dec 1997	
2.1.F4 Van de Giessen 1998	13	267	1998	
2.1.F5 Van de Giessen 1999	13	161	1999	
2.1.F6 Van de Giessen 2000	1	55	Jan-Mar 2000	
sum	35	599		0.0584

Table 3-8 gives the usable data at animal level. The overall animal level prevalence can be calculated using the average prevalence at farm level (0.058) and the animal level prevalence at negative and positive farms (0.0045 and 0.092, respectively). The overall animal level prevalence equals $0.058 \times 0.092 + (1 - 0.058) \times 0.0045 = 0.0096$.

Table 3-8 STEC O157 animal level prevalence data of dairy cattle at the farm. Negative and positive farms are farms previously found positive and negative, respectively. (Data from 2.1.F1 Heuvelink et al. 1998b, Heuvelink 2000d.) For negative farms, November measurements were averaged. For positive farms, September measurements were averaged and November 1997 measurements were not used. Animal level prevalence equals the no. of positive animals divided by the total no. of animals.

Farm	No. of animals positive	Total no. of animals	Animal level prevalence	Data from
Negative	3.5	777.5	0.00450	Nov 1996 - Nov 1997
Positive	64.5	702	0.0919	Sep 1996 - Sep 1997

The calculation for veal calves at the farm is shown below. Table 3-9 gives the usable data at farm level. The average prevalence at farm level can be calculated as $20/357 = 0.056$. There are no animal level prevalence data available for veal calves at the farm.

Table 3-9 STEC O157 farm level prevalence data of veal calves at the farm.

Study	No. of positive farms	No. of farms	Data from	Overall prevalence
2.1.F3 Heuvelink et al. 1999b	3	113	Apr - Dec 1997	
2.1.F4 Van de Giessen 1998	8	152	1998	
2.1.F5 Van de Giessen 1999	8	60	1999	
2.1.F6 Van de Giessen 2000	1	32	Jan - Mar 2000	
sum	20	357		0.0560

For the slaughterhouse, the availability of year-round data is very limited. No data on dairy cattle are available. Usable veal calve data are shown in Table 3-10. Herd and animal level prevalence are equal to zero.

Table 3-10 STEC O157 prevalence data of veal calves at the slaughterhouse. Data from 1997 - 2000 from 2.2.S7 Reinders 2000.

Level	No. positive	Total no.	Prevalence
Herd	0 herds	ca. 42 herds	0
Animal	0 animals	ca. 840 animals	0

The retail data are shown in Table 3-11. The average tartare prevalence is equal to $1/82 = 0.012$.

Table 3-11 STEC O157 prevalence data at product level for tartares at retail.

Study	No. of positive tartares	Total no. of tartares	Data from	Overall prevalence
2.3.R6 De Boer et al. 1997	1	39	1996	
2.3.R8 Heuvelink 2000a	0	43	1997	
sum	1	82		0.0122

3.2.2 Concentration

This section describes the data found on concentrations of STEC O157. A more extensive description is given in Appendix 2.5 - 2.7.

E. coli O157 counts in faeces

There are no Netherlands data available on *E. coli* O157 counts in faeces. Excluding data on experimentally infected cattle, we found two references. Shere et al. (1998) give a range of 200 - 87000 CFU/g faeces for *E. coli* O157:H7 in cattle. Zhao et al. (1995) gives *E. coli* O157:H7 - concentrations in the faeces of 31 positive calves from a survey of dairy herds in the U.S.. The results are presented in Table 3-12.

Table 3-12 Concentration of *E. coli* O157:H7 in faeces of calves from dairy herds in the U.S. Data from Zhao et al. (1995).

No. of samples	Concentration (CFU/g)
15	$< 10^2$
2	10^3
11	10^4
3	10^5

E. coli O157 counts on carcasses

There are no Netherlands data available. Daube and Wauters (1998) give two values for enterohemorrhagic *E. coli* O157 (EHEC O157) for Belgium: 1 and 200 CFU/cm².

E. coli O157 counts in tartare

There are no Netherlands data available for ground beef products and there are no foreign data available for tartare. Foreign data on ground beef products are shown in Table 3-13.

Table 3-13 Concentration of *E. coli* O157 in beef and beef products.

Product	Study	Concentration	Reference
Ground beef	Survey	0.4 - 1.5 cells/g	Padhye and Doyle 1991
Ground beef	Outbreak	0.3 - 15 MPN/g	Coleman et al. 1998
Hamburger patty	Outbreak	<700 per patty	Armstrong et al. 1996
Ground beef	Outbreak	500-1000 CFU/g	Armstrong et al. 1996
Beef	Other side of outbreak cow	100 CFU/g	Armstrong et al. 1996

3.2.3 Other

In this section attention is given to transmission of *E. coli* O157 and the source used for consumption data.

Transmission of E. coli O157 in slaughterhouse

No information was obtained on transmission of *E. coli* O157 in slaughterhouses, but it should be noted that literature on transmission of micro-organisms in slaughterhouses was not specifically searched for. A number of references were obtained on this subject, with data on *E. coli* in general or higher aggregation levels (Bell 1997, Chapman et al. 1993, Donkersgoed et al. 1997, Gill et al. 1996, Gill CO et al. 1998). See Appendix 2.8 for a short summary of these references. Usable interpretation of these data will require further study.

Consumption data

The third Dutch nutrition surveillance (Kistemaker et al. 1998) will be used as source for consumption data. The research for this database was done in the period April 1997 to March 1998 by GfK PanelServices Benelux by order of the ministries of Agriculture, Nature and Fisheries and of Public Health, Welfare and Sports. The database gives information on consumption behaviour and differences between population groups. It is based on a representative sample of 6250 persons living in 2564 households. Using diaries, participants register for two days what is eaten and drunk at home and abroad; days are spread over the year and the week. Through means of a separate questionnaire, data on living habits, body weight and a large number of personal and household characteristics are recorded for the participants. The information from the diaries is coded according to the food codes of the Dutch nutrient database, the so-called NEVO-table (Voedingscentrum 1996), resulting in a database which gives insight into the consumption of foods.

3.2.4 Food handling data

3.2.4.1 Information obtained prior to the expert workshop

An important part of food handling data was obtained from the Inspectorate for Health Protection and Veterinary Public Health, prior to the expert workshop. The information from this interview is presented below, subdivided into slaughtering, meat processing, tartare and retail.

Slaughtering

The steps taken during slaughter can be summarised as follows:

- visual inspection by a veterinary surgeon from the National Inspection Service for Livestock and Meat (RVV)
- stunning
- killing (by cutting an artery)
- dehiding
- cut open from arse to abdominal cavity (at this stage faecal contamination is judged)
- evisceration
- slaughtered inspection: quality of lungs, liver, kidneys

- dividing the carcass in two halves
- cold storage of the carcass

A cattle butcher differs from a slaughterhouse in the number of cattle slaughtered per time unit. Damaging the intestines, which results in faecal contamination, will occur less frequently with the cattle butcher. Also, he has more traditional insight in judging and increasing (by cleaning) the suitability of a carcass for consumption. On the other hand, the slaughtering process is less controlled than in a slaughter house. Cross contamination during slaughtering occurs via hands, tools, machines and by contact between carcasses. The amount of grams of faeces on a carcass is larger than zero for every carcass, but a maximum cannot be given. It is not allowed to clean carcasses with water (as this only spreads the micro-organisms) or e.g. H₂O₂, glyoxyl, ozone. Visual contamination with faeces must be cut away with a knife.

Meat processing

The difference between tartare and minced meat is that they have a fat content of maximum 10 % and 35 %, respectively. In industrial processing, certain technical parts are reserved in advance for tartare (based on production planning) and the meat chips (usually very limited in number) are used for minced meat. The traditional butcher (“cattle” and “carcass”) collects during cutting meat chips that are lean enough to be used for tartare. In addition, depending on the sale and the particular carcass at hand, technical parts can be selected for tartare. There is time to make a balanced decision on this. The larger part of tartare will be produced using meat chips.

In industrial processing, meat for tartare originates usually from certain technical parts. These are parts from the fore quarter (= shoulder + foreleg) and from the hind quarter (e.g. thick flank (usually used for steak) and silverside). These are parts that are also used for frying steaks and consist of tough, lean beef. For steak, more tender meat is used and it is more expensive. The technical parts used for tartare are partly at the surface of the carcass and partly not exposed to the surface. Meat chips originate from surface parts as well as non-surface parts.

Technical parts are stored at a temperature of 0-7 °C. The time period from cutting to further processing is two days maximum. The time period from cutting to purchase by the consumer is 2-6 days (depending on processing conditions). This is about meat without additives which is not gas-packed.

Tartare

In industrial processing, the production of tartare is done at the industrial deboning plant, not at a later stage in the food production chain. The size of the technical parts and meat chips used for tartare is 2-4 kg and 10-50 g, respectively. The traditional butcher collects meat chips and technical parts to an amount of 1-4 kg before processing to tartare. This amount of meat will originate from a limited number of animals. In industrial processing, meat processing will be batchwise.

The production of tartare consists of two steps:

- 1) freezing partially;
- 2) grinding once (prior to grinding of e.g. minced meat).

In industrial processing, freezing is done for a few minutes. A traditional butcher stores the meat in the freezer for a few hours maximum, such that the meat is not entirely frozen.

Retail

The traditional butcher sells tartare per piece or per amount. The supermarket butcher sells them pre-packed, with more than one in a package. The weight of one piece of tartare is 100 g and relatively constant. If more than one piece of tartare is pre-packed together, these originate from the same batch.

3.2.4.2 Information obtained as follow-up to the expert workshop

As follow-up to the expert workshop (see section 3.3.4.1), experts were consulted with specific questions. These are experts that took part in the workshop, but also other experts, from RVV, PVE and Company board butchers. The information obtained is presented below.

Slaughterhouse classification

RVV classifies slaughterhouses into 'article 4' and 'article 10' slaughterhouses. This is an EU recognition based on public health considerations. Article 4 slaughterhouses have a small capacity of 20 cattle per week maximum. They only distribute inland. They are only allowed to deliver directly to consumers or to institutions, not to third parties, such as a supermarket chain or a deboning plant. They take care of the whole process from slaughtering up to and including tartare production. Article 10 slaughterhouses have no capacity limit. They sell to clients all over the world. They are allowed to deliver to third parties. They do not process the meat further than up to and including the making of parts. Tartare is made by another company. Small article 10 slaughterhouses that slaughter much cattle per week but actually are not geared for it, are the most dangerous in terms of public health.

The business board butchers use the terminology 'selfslaughtering butcher' which is not used by the RVV. Selfslaughtering butchers are part of article 4 slaughterhouses. A selfslaughtering butcher slaughters 1 to 5 cattle per week.

A third classification is based on B and Q recognition. Every factory has a B recognition, but there is also a Q recognition relating to ground meat. For this, samples are taken daily and analysed for *E. coli* as a standard and occasionally for *Salmonella*. The number of micro-organisms determines the sell by date of the ground meat.

A fourth classification is based on considerations of market and trade. Large slaughterhouses ($\geq 10,000$ slaughterings per year) have an EU obligation for classification. The classification is on fat content, sex, and the thickness of meat compared to bones.

Meat flows

One expert states that a good subdivision of meat flow for slaughtering is into 'article 4 slaughterhouses' and 'article 10 slaughterhouses' rather than into 'selfslaughtering butchers'

and 'industrial slaughter'. As only article 10 slaughterhouses are allowed to deliver to third parties, three meat flows to the butchers are obtained. This implies that article 4 and 10 flows remain entirely separate. The scheme is depicted in Figure 3-7. However, it should be realised that a selfslaughtering butcher who needs meat less than a whole carcass can also buy meat. Another expert states that whereas in the past a butcher would buy a whole or a half carcass, nowadays often specific technical parts are bought.

Proportion of cattle slaughtering for slaughterhouse types

In 1999, 17 % of slaughters was done in slaughter houses with less than 10,000 slaughterings per year (see also www.pve.nl). This includes a part of the art. 10 factories. The RVV could not provide numbers on this subject in the short term, possibly partly due to the food and mouth disease crisis which occurred during the time period in which investigations for this report took place.

Proportion of sale for traditional and industrial tartares

The product board has no specific information on steak tartare. Within 'household sales', tartare is part of ground meat. The main part of ground meat is nowadays sold via the supermarket, not via butchers. For ground meat in 1999, 78 % is sold via the supermarket and 22 % via a specialist shop and others (PVE and Voorlichtingsbureau Vlees 2000). For ground meat in 2000, based on data from bureau GfK, the numbers are 19 % via butcher, 79 % via supermarket and 2 % others (e.g. a warehouse with a supermarket department, or a poulterer selling ground meat).

A second expert could only give the general proportion for meat sale of 20 % for butcher and 80 % for supermarket. It is stated that this is the sale over the counter which does not include the back door sale, e.g. to institutions, and that selling figures are not reliable.

Import

One expert states that chilled meat meant e.g. for tartare production or tartare is imported from Ireland, Germany, Belgium, Austria, France and America. Another expert states that mainly Dutch meat is used, because cheap meat from e.g. Argentina and Brazil remains there; only the more expensive meat is exported to the Netherlands and this meat is not ground. A third expert states that much of the meat that is consumed in the Netherlands is of foreign origin.

Part of cattle processed to tartare

To estimate the part of cattle processed to tartare, data are derived by an expert from calculations of the butcher profession education (SVO). These are extrapolated numbers because they are based either on hind quarter or on fore quarter data. The percentages given in Table 3-14 deviate caused by change in demand for tartare, the meat can than be used for other products or extra meat will be necessary.

Table 3-14. Percentage of cattle used for tartare.

Type	Age (years)	Percentage
Female cattle	± 3	9.4
Steer	± 3	10.1
Limousin	2.5	8.2
Cow	4.5	9.7
Friesian (Holstein)	4	7.8

Meat processing

One expert states that a deboning plant delivers vacuum-sealed packs of 1-2 kg containing meat parts smaller than 100 g.

Tartare

An example of ground meat/tartare production given by an expert: a conveyer belt is used, meat comes in on one side and out on the other without being touched by human hands. Frozen pieces of meat of 35-40 kg are supplied, are made smaller, mixed and ground. Temperature must remain below 7 °C by law, but 4 °C is aimed at.

There is a ground meat guideline of the European Union. The Netherlands is the only country in Europe in which ground meat is finalised at a large scale for delivering to the consumer. Only in Switzerland this is also done at a small extent. In other countries, the butcher is asked to ground a piece of meat on the spot. Hygienically this is much more unfavourable.

Batch weight

An expert estimates the weight of an industrial batch ground beef to be at least thousands of kilos. Another expert gives an estimate of 180-200 kg for ground meat and tartare, being the size of a cart used in factories. A third expert states that a factory grinds once a week, and for ground meat this will be 100-200 kg per occasion and for tartare 25-100 kg per occasion. These amounts vary much. A fourth expert states that an example factory produces 5000 kg ground meat per day and 500 - 1000 kg tartare per day.

One expert estimates that a batch traditionally prepared ground meat and tartare will weigh 5-20 and 2-20 kg, respectively, depending on the trade. If higher amounts of stock are ground, then a grey discoloration caused by lack of oxygen will arise. Another expert estimates that a traditional butcher uses batches ground beef of 10 - 15 kg. A third expert gives a range for tartare of 0.5 kg (occasional) to 25 kg (considered very high) per grinding occasion.

3.3 Risk model definition

3.3.1 Introduction

At the start of the project, starting points were formulated that were to be used for the risk assessment modelling. These consist of modelling considerations and defining relevant phenomena or variables, and were based on the information that was available at the start of the project (see Chapter 2 to and Sections 3.1. and 3.2) The starting points are partly general and partly specific for different stages of the food pathway.

General starting points are:

- Start simple and extend later on;
- From a modelling point of view the Netherlands contains, one slaughterhouse, one butcher, etc.;
- Attention will be given to the dimensions of variables that are passed between stages of the food pathway;
- The model will contain no time dynamics;
- The MPRM approach will be used (see below).

Next, stage-specific starting points will be given below.

Farm

Modelling considerations:

- No input of STEC O157 into this stage from other compartments will be modelled;
- Data of STEC O157 will be used as a starting point for calculations;
- Possibly calves, adult cattle, etc. will be distinguished in the risk assessment;
- Even in the simplest modelling, not a point estimate but a distribution of the concentration of micro-organisms in the faeces will be used.

Transport

Relevant phenomena are cross contamination and stress.

Slaughterhouse

Modelling considerations:

- After slaughter only a specific part of the carcass is used for the specific product;
- The simplest modelling option will be an input-output model with prevalence and concentrations as variables;
- Hygiene of slaughtering determines to what extent faeces (containing micro-organisms) will end up on the surface of a carcass;
- A certain part of the micro-organisms on the carcass will end up on the final product.

Relevant phenomena are cross contamination, freezing, cleaning and microbial growth.

Retail

Relevant phenomena are partitioning, mixing, contaminated ingredients (possibly), temperature effects and cross contamination.

Household

Relevant variables for inactivation are refrigerator temperature, preparation temperature and ingredients. Relevant phenomena are partitioning, mixing and cross contamination.

From exposure to effect modelling

Important variables with respect to passing through from exposure to effect modelling:

- The number of micro-organisms on or in a product;
- The prevalence of contaminated products;
- The number of people that consume the product (possibly distinguish subpopulations);
- The frequency of consumption;
- The size of the product.

Public health

In the model distinguish at least children as a subpopulation, because of HUS.

As a modelling approach, the Modular Process Risk Model (MPRM) methodology is applied. This QMRA methodology has recently been introduced (Nauta 2001b, Nauta 2001a). In a MPRM the transmission of a hazardous micro-organism along a food pathway is modelled by describing the changes in prevalence (P) and number of micro-organisms per unit (N). Characteristically, the food pathway is split up in consecutive modules, which are to be considered as one of six basic processes, that is either one of two microbial processes (growth and inactivation) or one of four food handling processes (mixing, partitioning, removal or cross contamination). If a process is too complex to be modelled as such, a 'black box model' may be applied.

The modelling of the basic processes has been discussed previously (Nauta 2001b). In this report we therefore only give a short introduction to the modelling of each of the basic processes. (As a new method to include non-random distribution in partitioning and mixing process is introduced here, these two discussed in more detail) (3.3.2). The specific models used for each module (the process steps) are discussed more precisely (3.3.3), followed by an account for the parameter estimates (3.3.4).

3.3.2 Basic processes

3.3.2.1 Growth and inactivation

Microbial growth and inactivation are typical microbiological processes leading to either an increase or a decrease in the population size (that is the number of micro-organisms per unit).

Predictive microbiology models offer a wide scope of models to quantify growth and inactivation, as a function of time, temperature, acidity, water activity *etc.*, and for a variety of microbial species (e.g. McMeekin et al. 1993, Whiting 1995, Van Gerwen and Zwietering 1998). Traditionally, variability in growth and inactivation is not incorporated in these (deterministic) models. As for calculation of risks one is not so much interested in point estimates of population sizes, but in the probability of reaching different population sizes, the use of these predictive models in QMRA models is not obvious (Nauta 2001a).

As explained in 3.3.3., growth and inactivation are to be modelled at three stages along the food pathway in this study: during the time between carcass halving and carcass trimming (step 3), during storage after tartare production (step 7) and during preparation of tartare patties (step 8). It was not possible to use the same growth/inactivation model throughout the risk model: due to an overall lack of (sufficient) knowledge on both crucial process characteristics like process time and temperature, and pathogen behaviour on the specific substrates considered, it was decided to use a pragmatic approach. The mode choice depended on the available data and models for similar processes as used in the literature.

3.3.2.2 Partitioning

Partitioning occurs when a major unit is split up into several minor units, as given schematically in Fig 3-4.

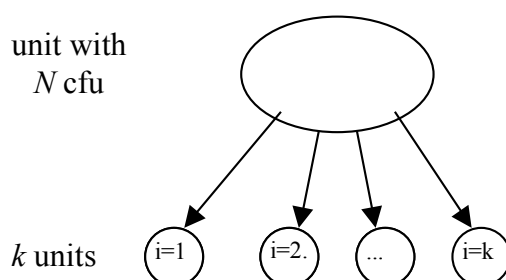


Figure 3-4 Partitioning: A major unit, containing N cfu (particles, spores, cells, etc) is split up in k smaller units i ($i= 1..k$).

Assuming random distribution and equal sized smaller units, sampling leads to a number of cells N_i' as a sample from a Binomial ($N, 1/k$) distribution for one smaller unit i . So the expected number of cells is N/k . If the smaller units are not equal sized, and the smaller unit has size m_i compared to size M of the major unit, $N_i' \sim \text{Binomial}(N, m_i/M)$ for this one smaller unit.

For a series of k equal sized smaller units i , there is a problem of dependence between the samples. The sum of the cells in all smaller units should equal the total N . Also, it is preferred that the probability distribution of the number of cells is the same in each smaller unit. It can be derived that this problem is solved by applying (Nauta 2001b and Appendix 3):

$$N_i' \sim \text{Binomial}[N - \sum_{j=1}^{j=i} N_j', 1/(k-i+1)] \quad (3.1)$$

(here \sim means: “is a random sample from”.) Micro-organisms will often occur as clusters of cells. On solid substrates like a carcass surface, random distribution of cells may be improbable. To deal with this clustering effect equation (3.1) above is adapted by considering that the probability that a cell will be found in a smaller unit is not equal for each smaller unit. This probability is variable with a Beta($b, b(k-1)$) distribution, with b a clustering parameter. (Note that the mean of this distribution is $1/k$, as it should be). When b approximates zero, the clustering effect is at its maximum with all N cells ending up in one smaller unit. When b is infinitely large, there is no clustering effect. Now (3.1) becomes a Betabinomial distribution function (see Appendix 3),

$$N_i' \sim \text{Binomial}[N - \sum_{j=1}^{j=i} N_j', \text{Beta}(b, b(k-i))] \quad (3.2)$$

so that (3.1) equals (3.2) when b is infinitely large.

In this risk assessment partitioning occurs at several steps in the food pathway: at carcass halving (step 2), with trimming (step 4), and when the tartare patties are produced from the ground beef batch (step 6). In each instance, there is a smaller mass m , that originates from a larger mass M . Then for k in equation (3.2), $x = M/m$. When m varies for different smaller units i , k varies for different units i too. In that case x in (3.2) is substituted by k_i with $k_i = M/m_i$.

3.3.2.3 Mixing

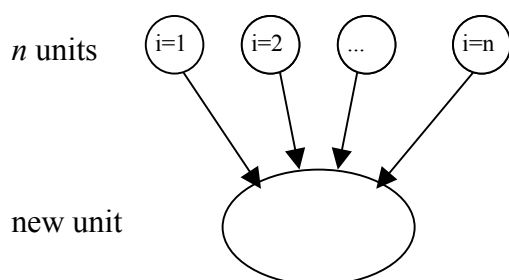


Figure 3-5 Mixing: n units, containing N_i cfu (particles, spores, cells, etc) in all n units i are put together to form a new larger unit. This larger unit will contain $N' = \sum_n N_i$ cfu. We want to know the distribution of N' , given the distribution of N_i .

Mixing is the opposite of partitioning, in which units are gathered to form a new, larger, unit as shown in Fig 3-5. In this new unit the number of micro-organisms is the sum of the number in all units it originates from:

$$N^{\circ} = \sum_i N_i \quad (3.3)$$

With mixing, the number of micro-organisms per unit (N_i), the size of the large unit, and both the number and sizes of the units mixed may be variable. If a large unit has size Q , and k smaller units i with size q_i contribute to it (such that $\sum_i q_i = Q$, with $i=1..k$), it may be that all smaller units contribute equally, such that $q_i = Q/k$. This is the case when all smaller units have the same size. However, the sizes of the smaller units may be variable too. If Q is known, the probability distribution function used to describe the variability of these smaller units has to take account of the fact that the sum of samples is fixed, that is that the samples are not independent.

In modelling mixing we introduce a methodology to account for unequal contributions of the different units added. As an example, think of the faeces that contaminates a carcass. If several animals in a slaughter line contaminate a carcass, they probably will not contribute equally to the total faecal contamination. The amount of faeces q_i will not be equal for each animal i . To determine the relative contribution of each animal, we need a method to divide the total faecal contamination (of Q grams) into k partitions q_i , such that each q_i is a sample from the same distribution, and that all k q_i sum up to Q .

The methodology chosen is comparable to the methodology to model clustering in partitioning (see 3.3.2.2.) As shown in Appendix 3, if k units are added, the relative contribution of a unit (that is the fraction of the sum coming from that unit) can be given by a Beta distribution $\text{Beta}(b, b(k-1))$. For a series of k units i ($i=1..k$), the following algorithm can be applied:

unit 1 is a sample from $\text{Beta}(b, b(k-1))$: x_1 ;

The size of unit 1 is $q_1 = x_1 Q$

unit 2 is a sample from $\text{Beta}(b, b(k-2))$: x_2 ;

The size of unit 2 is $q_2 = x_2 (1-x_1) Q$

...

unit y is a sample from $\text{Beta}(b, b(k-y))$: x_y ;

The size of unit y is $x_y \prod_{i=1}^{y-1} (1-x_i) Q$

unit k has $\prod_{i=1}^{k-1} (1-x_i) Q$

with b a parameter referred to as the "beta factor" ($b > 0$). If b is infinitely large all units have the same size ($1/k$), if b approximates zero, one unit has relative size 1, and all other units have relative size 0.

In this study, mixing occurs in two steps. Mixing is part of the carcass contamination process, when it is assumed that faeces on a carcass originates from more than one animal (step 1), and is the basic process when trimmings are grounded for the ground beef batch (step 5).

3.3.2.4 Other basic processes

Removal and cross contamination are not used as described in Nauta 2001b.

The carcass contamination (step 1) is a complex process, comprising initial contamination, cross contamination and mixing. Below it is modelled as one process.

3.3.3 Modelling the Food Pathway

In consecutive steps the transmission of STEC O157 is described by modelling the change in the number of micro-organism per unit N . The whole pathway is given schematically in Fig 3-6. In the model of the food pathway, the ground beef batch is the central unit. A run of the Monte Carlo model simulates the production of a ground beef batch and the consecutive production and consumption of tartare patties derived from that ground beef batch. At each step, the number of micro-organisms at the end of the process (N') is given as a function of the number in the previous step (N).

Due to the complexity of the food pathway, three different routes of exposure were considered, which required separate estimates of some of the model parameters. This is illustrated in Fig 3-7. At first a differentiation was between different types of slaughterhouses. As explained in sections 3.1 and 3.2.4, a large variety in slaughtering practices occurs in the Netherlands. The number of animals slaughtered in a slaughterhouse may vary from 1 to several thousands of animals per week, the meat may be for the internal market, for export, and both, *etc.* Based on comments from the expert panel (see 3.3.4.1.) we make a distinction between two types of slaughterhouses, according to EU regulations: Article 4 and Article 10 slaughterhouses (see 3.2.4.2.). A further differentiation is between traditional tartare production by the butcher, and industrial tartare production at the slaughtering plant. The quantities of steak tartare produced by the butcher (and thus the size of their ground beef batches) are considerably smaller.

At the level of exposure of the consumer, for a proper connection with the effect modelling, we had to differentiate between three age classes (*ac*): 1-4, 5-14 and 15+ years, and three tartare patty preparation styles (*pst*). Tartare patties can be eaten raw, and 'medium' and 'well done' when cooked. Finally we needed information on the number of inhabitants of different age classes in the Netherlands, to quantify the population burden in number of cases per year in the Netherlands.

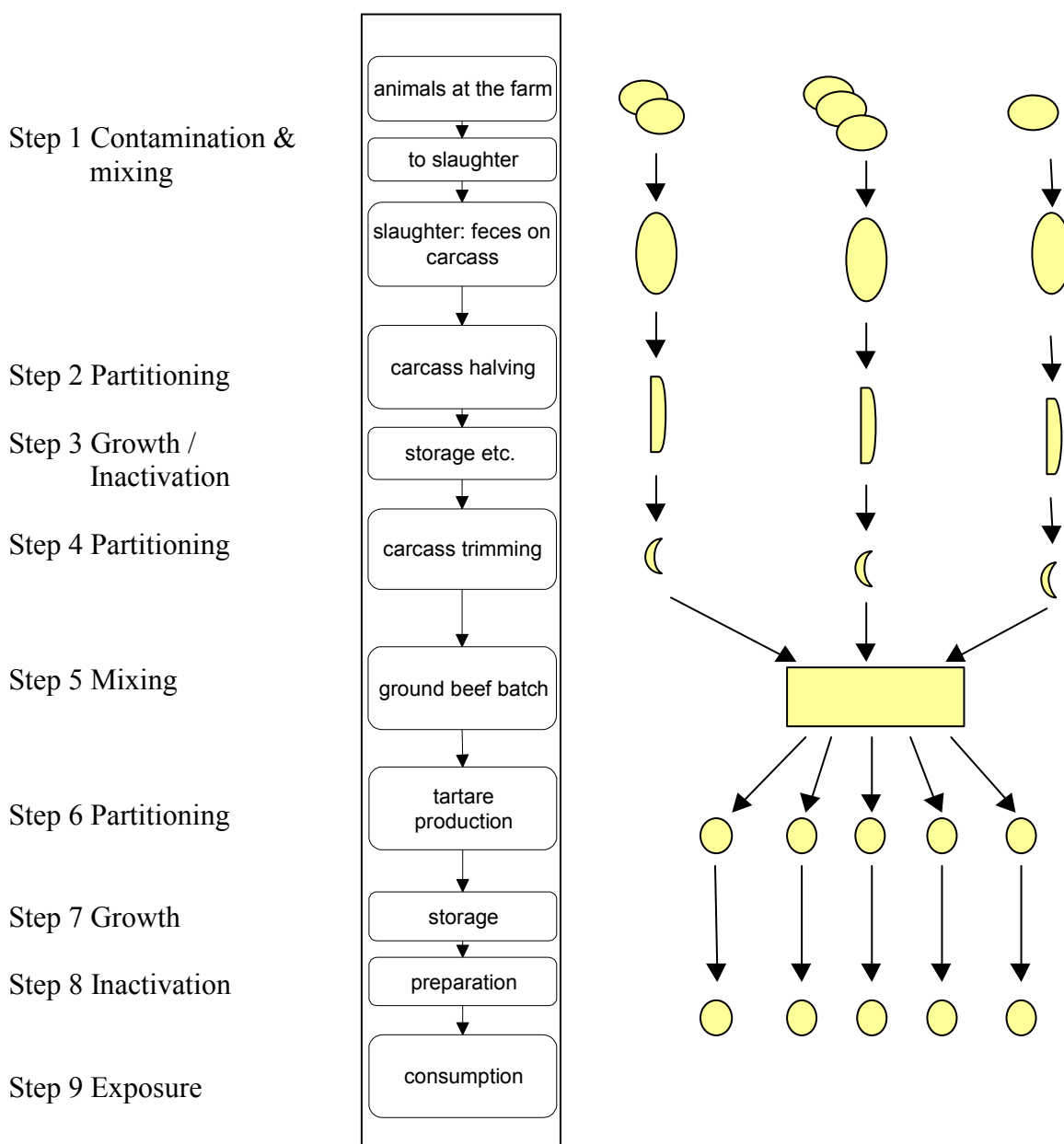


Figure 3-6 A schematic representation of the Food Pathway as modelled in the exposure assessment. From top down m_i animals (lying ovals) contaminate the n carcasses i (hanging ovals), which after halving and trimming contribute meat to the ground beef batch (rectangle). From the ground beef batch tartare patties are produced (circles).

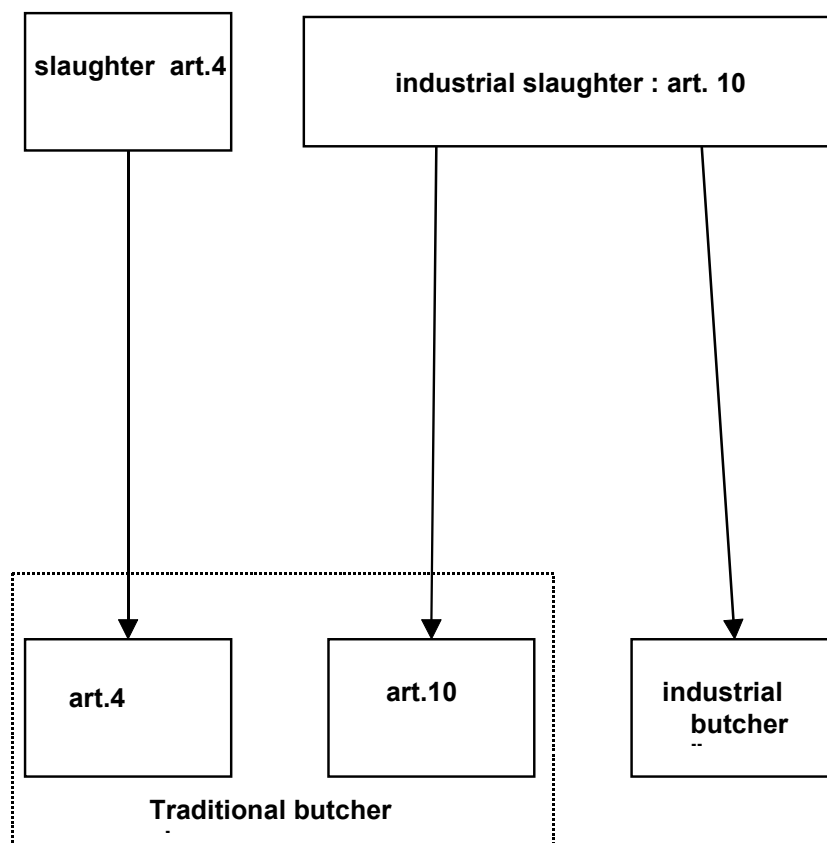


Figure 3-7 Routes of exposure as modelled in the risk assessment. Two types of slaughterhouses produce meat for two types of butchers. For explanation see main text.

Step 1: Contamination of carcasses

The prevalence of contaminated farm animals (defined here as the animals shedding faeces containing STEC O157) is P_f . This is an uncertain parameter, which is variable in time. As time is not a dimension in the model, P_f is assumed to be constant throughout this model. Its impact on the risk model result is studied in the scenario analysis.

We assume that trimmings from n (half) carcasses are contributing to the ground beef batch of W_{gb} kg. Next, we assume that faeces from m_i animals ($i=1..n$) contributes to the faecal contamination of the carcasses i . Parameters n and m_i will be variable per ground beef batch produced. This variability is implemented as

$$n \sim 1 + \text{Poisson}(n_{\text{mean}}-1)$$

$$m_i \sim 1 + \text{Poisson}(m_{\text{mean}}-1)$$

With these equations n and m have a discrete value with a minimum of 1 and a probability density function characterised by one parameter only.

In total, this approach implies that $M = \sum_i m_i$ animals contribute to the (possible) contamination of the ground beef batch ($i = 1..n$).

The total number of cfu on a carcass is a function of

- the fraction of the faeces that animal j contributes to carcass i . The relative contribution of each of the animals to the total amount of faecal contamination is described by a Beta distribution (see 3.3.2.3. and Appendix 3):

$$f_{ij} \sim \text{Beta}(b_1, b_1 (m_i-1)),$$

with 'beta factor' b_1

- the concentration of STEC in the faeces of animal j , contaminating carcass i : C_{ij} (cfu/g), noting that with probability P_f $C_{ij} > 0$ and with probability $1 - P_f$ $C_{ij} = 0$.

- the total quantity of faeces on carcass i (in g) $a_i \sim a_{\max} \times \text{Beta}(\alpha, \beta)$ with a_{\max} , α and β model parameters expressing the level of faecal contamination and its variability per carcass. The choice for the Beta distribution to describe the faecal contamination was made after fitting the results of expert estimates on this topic to a series of probability distributions, as shown in 3.3.4.

The expected number of cfu on carcass i is now $E(N_i) = a_i \sum_j f_{ij} C_{ij}$. The simulated number N_i has to be an integer. Therefore $N_i \sim \text{Poisson}(E(N_i))$.

Note that a basic assumption here is that animals, faeces and concentration are all independent, i.e. the presence of a STEC contaminated animal does not affect the probability of contamination of another one (e.g. from the same farm). This simplifying assumption may not be realistic, but it probably has little impact on the results of the risk assessment.

Step 2: Partitioning to half carcasses

The carcass is split into two. The weight of the clean carcass is W_{carc} and the weight of the half carcass is $0.5 W_{\text{carc}}$. This is partitioning with $x = W_{\text{carc}} / 0.5 W_{\text{carc}} = 2$ and $N = N_i$, so $N_i \sim \text{Binomial}(N_i, \text{Beta}(b_2, b_2))$, with b_2 a parameter describing the clustering at carcass halving.

In the Monte Carlo simulation we use one half carcass only. The other half is neglected.

Step 3: Growth and inactivation on the carcasses

Although beef carcasses are chilled during storage, conditions for different STEC O157 on a carcass will be variable. As indicated by Cassin et al. (1998), the concentration of STEC O157 on 'clean' carcasses may both increase as decrease. To describe the aggregate proliferation on a carcass surface, they use a parameter G_{PRC} (microbial growth during processing) with an assumed triangular distribution with minimum -2, mode 0 and maximum 5 generations proliferation. This assumption is derived from observations on pig carcasses.

We have no data on this for the Dutch slaughtering practices. During the expert workshop, the experts assessed the equivalent minimum, most likely and maximum value for G_{PRC} , typical for the Dutch situation .

Step 4: Partitioning to trimmings

Trimmings are cut from the carcasses, obviously a partitioning process. To assess the number of STEC O157 on the trimmings from one half carcass used for the ground beef batch, we use the number (N_i) on each half carcass i , and the weight of these trimmings from this half carcass (W_{tri}). This weight is determined in the mixing process in step 5 below. (Note that W_{tri} is not the weight of one trimming, but the total weight of all trimmings from carcass i .)

To describe the number of STEC O157 on the trimmings from half carcass i used in the ground beef batch, we use the Betabinomial distribution function:

$$N_i' \sim \text{Binomial}(N_i, \text{Beta}(b_4, 0.5 W_{carc} b_4 / W_{tr,i} - 1)), \quad (3.4)$$

with b_4 a parameter describing the clustering effect of cells on the carcass when trimmings are cut off.

We thus assume that the probability of finding STEC O157 on meat destined for tartare is equal to the probability of finding it at a random place on the carcass. As clustering is incorporated in the model, the cells are not assumed to be spread equally over the carcass. However, in the model the probability of finding a cluster is not related to the type of meat used for tartare.

Step 5: Mixing: the ground beef batch

When the ground beef batch is formed, it contains meat of n animals. The total weight of all the trimmings from one (half) carcass used for the ground beef batch depends on the number of carcasses used for the batch (n), and the weight of the batch (W_{gb}).

The carcasses need not contribute equally to the ground beef batch. We therefore use the algorithm as outlined in 3.3.2.2. and Appendix 3, such that for a random half carcass i

$\phi_i \sim \text{Beta}(b_5, b_5(n-1))$ and $W_{tr,i} = \phi_i W_{gb}$ with ‘beta factor’ b_5 (see Appendix 3).

Implementing the distribution of the $W_{tr,i}$ in equation (3.4) of step 4, we calculate

$$N' = \sum_n N_i.$$

Step 6: Partitioning to tartare patties

Tartare patties are produced from the ground beef batch. This is a typical partitioning process. Therefore the number of STEC on tartare patty j is:

$$N_j' \sim \text{Binomial}(N, \text{Beta}(b_6, W_{gb} b_6 / W_{tt,j} - 1)) \quad (3.5)$$

with b_6 a parameter describing the clustering effect of cells in the ground beef batch for tartare patty formation. This clustering effect may indicate the clustering of ‘not well mixing’ a large ground beef batch.

The weight of the tartare patty will vary, with a different distribution per age class. For these distributions of weights we use information from the Dutch nutrition surveillance (Kistemaker et al. 1998), as explained in 3.3.4.

Step 7: Growth of *E coli* during tartare storage

Several authors have developed models for the growth of *STEC O157* as a function of growth conditions like temperature, acidity, water activity, etc. (e.g. Buchanan and Klawitter 1992, Buchanan and Bagi 1994, Presser et al. 1997, Nauta and Dufrenne 1999). The data from Buchanan and Bagi (1994) are used for the USDA Pathogen Modelling Program (PMP, USDA 1998), which is available on the internet. Walls and Scott (1996) validated this model in raw ground beef and concluded that the PMP appears suitable for use with raw ground beef. Nevertheless, they observed some differences, primarily shorter lag phases at pH 6.3 at 12°C.

In this study we used the PMP to derive models for growth of *E. coli* O157 in steak tartare, expressed in two equations, expressing the generation time (GT) and lag phase duration (LPD) as a function of temperature. As typical characteristics for tartare ‘pH 6.2, NaCl 1%, no NaNO₂’ were used (R. Reinders, *pers. comm.*) At those conditions, in the PMP the LPD and the GT are recorded between 5°C and 20°C. (Using the equations given in the paper of Buchanan and Bagi (1994) gave unrealistic predictions, incompatible with the PMP, probably due to some misprint(s).) As illustrated in Fig 3-8 the PMP data (USDA 1998) fitted well to the following equations.

$$GT(T) = \frac{1}{(0.0295T + 0.356)^{4.24}} \quad (3.6)$$

and

$$LPD(T) = \frac{1}{(0.021T + 0.294)^{5.88}} \quad (3.7)$$

These equations are used to predict the expected increase in population size growth in tartare as a function of storage time and temperature:

$$E(\log(N_j')) - \log(N_j) = \log(2)/GT(T) \times (t-LPD(T)) \quad (3.8)$$

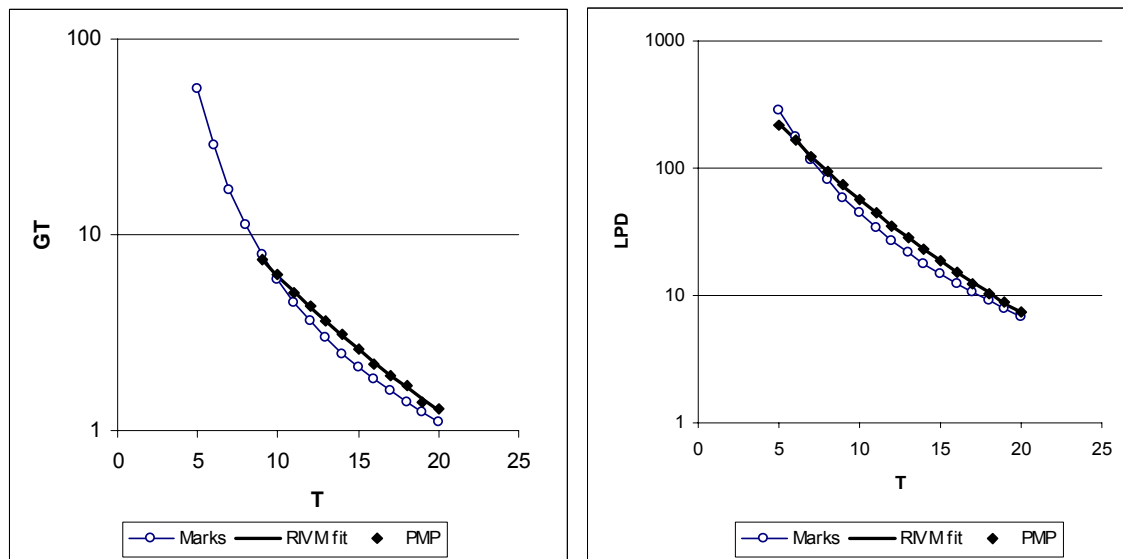


Figure 3-8 The relation of Lag phase duration (LPD) and Generation time (GT) of STEC O157 in tartare and temperature, in the relevant interval between 5 and 20 °C. Data from the PMP (USDA 1998) are fitted with the equation (3.6) and (3.7) (RIVM fit). The growth relation used by Marks et al. (1998) for a risk assessment study of *E. coli* O157:H7 in hamburger is given for comparison.

To account for stochasticity in the growth process, incorporating that N' should be a whole number, the predicted number of *E. coli* O157 cfu in one tartare after storage is calculated as a random sample from a Negative Binomial distribution (Nauta 2001b):

$$N_j' \sim N_j + \text{NegBin}(N_j, N_j/E(N_j'))$$

Storage temperature and storage time between the moment of tartare production and preparation of tartare, T and t , may be variable. It is assumed that the variability of the temperature per steak tartare patty has a normal distribution with mean T_{mean} and standard deviation T_{sd} . Time is assumed variable per steak tartare patty with an exponential distribution with mean t_{mean} .

Step 8: Inactivation during preparation of patties

Thermal inactivation of bacteria is commonly modelled with the Bigelow model, characterised by a D-value and a z-value. Several authors report D- and z-values of *E. coli* O157 in ground beef (Doyle and Schoeni 1984, Line et al. 1991, Ahmed et al. 1995, Jackson et al. 1996). According to Ahmed et al. (1995) and Line et al. (1991) inactivation depends on fat content (higher fat content gives higher D-values, i.e. less inactivation). Jackson et al. (1996) study the effect of storage temperature on inactivation: Cold storage decreases the inactivation effect, warm storage increases this effect. They state that 54.4°C endpoint

temperature is typical for rare hamburgers, 62.7°C is typical for medium and 68.3°C is typical for ‘well done’.

However, to apply the Bigelow model in risk assessment, the inactivation temperature profile and the effects of non-isothermal inactivation have to be known. Unfortunately we have no data on heating temperatures of steak tartare patties in Dutch households. D- and z-values can therefore not be used directly.

According to the model used by Juneja et al. (1997), as applied by Cassin et al. (1998) the log reduction in hamburgers as a function of the internal temperature is given by $y = -10.165 + 0.211 T$ (in °C) log cfu. This implies for an internal tartare temperature of resp. 50,60,70 and 80°C, 0.5, 2.5, 4.6, and 6.7 log reductions. Relating ‘doneness’ preferences with data on internal temperatures of hamburgers (Jackson et al. 1996), Cassin et al. (1998) assess the inactivation in different types of hamburger. Tartare patties are, however, no hamburgers. Tartare has a lower fat content, patties are thicker, and may be consumed raw. When cooked, the internal part of the tartare patty should still be red. We are therefore not able to translate the ‘hamburger’ methodology’ of Cassin et al. (1998) to our risk assessment directly. Instead, the experts at the experts workshop were asked to estimate the effect of inactivation during preparation, given the information on inactivation of hamburgers as outlined above.

Roughly, it is assumed in our study that a proportion of the tartares is consumed raw, a proportion is consumed ‘medium raw’ (red internally) and a proportion is consumed ‘well done’. Each treatment results in an inactivation/survival effect f_{surviv} , such that $f_{\text{surviv}} = 1$ represents no inactivation, $f_{\text{surviv}} = 0$ represents total inactivation and for example $f_{\text{surviv}} = 0.01$ represents 1% survival.

Dealing with stochasticity (Nauta 2001b), with an expected number of cfu after inactivation is $E(N_j') = f_{\text{surviv}} N_j$,

$$N_j' \sim \text{Binomial}(N_j, f_{\text{surviv}}).$$

This N_j is the ingested dose when tartare patty j is eaten.

Step 9: Exposure at consumption

To assess the exposure at population level, we need to include the number of tartares eaten per year, and tartare consumption frequencies per age class (1-4, 5-14 and 15+) and per preparation style (‘raw’, ‘medium’ and ‘well done’).

We therefore define $P_{\text{cons,ac,pst}}$ as the probability of consumption of a tartare (per day) prepared with preparation style pst , for a person in age class ac . It is assumed that no more than one tartare is eaten per day. Per age class ac and preparation style pst the prevalence of contaminated tartare patties is $P_{\text{pos tt,ac,pst}}$, as derived from the Monte Carlo simulation. The per day probability of ingesting STEC 0157 for a person in age class ac is

$\sum_{\text{pst}} P_{\text{pos tt,ac,pst}} \times P_{\text{cons, ac,pst}}$. The number of cells ingested is N_j ($N_j > 0$) as found in the Monte Carlo simulation.

Table 3-15 The production process and the model of the dynamics of the number of cfu per unit. part. = partitioning, gr. = growth, ina. = inactivation, mix. = mixing

step	unit	process	N (cfu/unit)	unit size
1			<p>The prevalence of animals with faeces positive for STEC is P_f</p> P_f <p>n carcasses are contaminated with a_i g faeces of m_i animals, with a concentration C_{ij} cfu STEC/g ($i=1..n, j=1..m_i$).</p> $n \sim 1 + \text{Poisson}(n_{\text{mean}}-1)$ $m_i \sim 1 + \text{Poisson}(m_{\text{mean}}-1)$ $a_i \sim a_{\text{max}} \times \text{Beta}(\alpha, \beta)$ $\log C_{ij} \sim \text{RiskCumul}(0,6, (2,3,4,5), (0.469, 0.531, 0.875, 0.969))$ <p>The relative contribution per animal is f_{ij}.</p> $f_{ij} \sim \text{Beta}(b_1, b_1 (m_i-1))$ <p>The expected number of cfu per carcass is $E(N_i) = a_i \sum_j f_{ij} C_{ij}$.</p> <p>carcass i $N_i' \sim \text{Poisson}(E(N_i))$ W_{carc} kg</p>	
2	half carcass i	part.	$N_i' \sim \text{Binom}(N_i, \text{Beta}(b_2, b_2))$	$0.5 W_{\text{carc}}$ kg
3	half carcass i	gr./ina.	$N_i' = N_i \times 2^{G_{\text{prc}}}$ (rounded to integer)	$0.5 W_{\text{carc}}$ kg
4	trimmings	part.	$N_i' \sim \text{Binom}(N_i, \text{Beta}(b_4, 0.5 W_{\text{carc}} b_4 / W_{\text{tri},i} - 1))$ with $W_{\text{tr},i} \sim \text{Beta}(b_5, b_5 (n-1))_i W_{\text{gbb}}$	W_{tri} kg
5	ground beef	mix.	$N' = \sum_n N_i$	W_{gbb} kg
6	tartare	part.	$N_j' \sim \text{Binom}(N, \text{Beta}(b_6, b_6 W_g / W_{\text{tt}} - 1))$ (per age class)	W_{tt} kg
7	tartare	gr.	$N_j' = 10^{(\log(N_j) + f(T, t))}$ $T \sim \text{Normal}(T_{\text{mean}}, T_{\text{sd}})$ °C $t \sim \text{Exponential}(t_{\text{mean}})$ h (per age class)	W_{tt} kg
8	tartare	ina.	$N_j' \sim \text{Binomial}(N_j, f_{\text{surviv}})$ (per age class and preparation style)	W_{tt} kg
9			$N_j' = N_j$ Prevalence tartare patties per age class and preparation style: $P_{\text{tt, ac, pst}} = P_{\text{cons, ac, pst}} \times P_{\text{pos tt, ac, pst}}$	

3.3.4 Estimation of parameter values

The next step is to assign values to the parameters defined in the model outlined above (3.3.3). For this purpose, several data sources are applied. The first option was data from international scientific journals, the second was other published data (written or electronic) from (for example) agricultural/veterinary/food manufacturing organisations, and the last option was the use of experts, who might open data sources that were not available to us, or, if not available, give their expert opinion as a parameter estimate. We were urged to use the last option for a set of sixteen parameters as the data outlined in 3.2 did not cover the parameters. Therefore, an expert elicitation workshop was organised at RIVM, in which nine experts participated. This workshop and the resulting parameter estimates is discussed below (3.3.4.1)

Next (3.3.4.2) we account for the model parameter estimates along the food pathway. Note that uncertainty in the parameter estimates is not included. All probability distributions described below represent variability (per ground beef batch produced) .

3.3.4.1 *The expert workshop*

In the risk assessment model we defined several parameters for which we were not able to estimate the value on the basis of literature data. Therefore an expert elicitation workshop was organised, with nine experts from different areas of knowledge. The participating experts were:

- E. de Boer (food microbiologist) from the Inspectorate for Health Protection and Veterinary Public Health, Zutphen;
- B.W. Ooms (specialist meat and meat products) from the National Inspection Service for Livestock and Meat, Voorburg;
- A.M. Henken (veterinary epidemiologist) and F.M. van Leusden (food microbiologist) from the National Institute of Public Health and the Environment, Bilthoven;
- G. Keizer (meat technologist) from the Netherlands Organisation for Applied Scientific Research, Zeist;
- L.J.A. Lipman (veterinarian) and R.D. Reinders (veterinary microbiologist) from Utrecht University;
- J.M. Schouten (veterinary epidemiologist) from Wageningen University;
- P.H.M. Janssen (mathematician, expert in expert elicitation methodology) from the National Institute of Public Health and the Environment, Bilthoven.

These experts met in an expert meeting organised on 30 January 2001 at RIVM Bilthoven. During a three hour plenary session, after a short introduction, the experts were asked to estimate the values of 15 model parameters. Each parameter was explained plenary, after which the experts got the opportunity to raise questions. Each expert gave an individual estimate of each parameter on an expert enquiry form. We not only asked for an estimate of the 'most likely value' of each parameter, but also for minimum and maximum values. A complicating aspect here was that for some parameters this minimum and maximum had to

reflect variability, and for others it had to reflect uncertainty. When reflecting variability, the minimum and maximum should be values that are the extremes of what the experts think to occur in reality. When reflecting uncertainty the expert should express the extremes of its belief on what might be the real value. Although the workshop organisers tried to explain this difference, a lack of familiarity of the experts with these matters, combined with the moderate level of educational skills of the workshop organisers, may have been the source of some misunderstandings.

To weigh the expertises, each expert was asked to indicate its expertise per parameter by checking a box, on a scale of five classes ranging from 'no knowledge' to 'high expertise'.

For most parameters the experts could give their opinion by a direct estimate of the parameter value. For the effect of clustering, however, this was problematic, due to the complex interpretation of the clustering parameter b . Therefore the experts could select the estimated clustering effect by selecting a minimum, most likely and maximum pie chart diagram (as in Appendix 3) representing the expected distribution of cells over the units. These pie charts were then translated to parameter values by the authors.

The expert parameter estimates are given in Table 3-16.

The individual estimates of the experts were summarised to an average 'expert panel' estimate for each parameter. This was done using the methodology of (Vose 2000). Per expert the minimum, most likely and maximum values as estimated were implemented in a BetaPert(min, ml, max) distribution. The expertise weights as indicated by the experts were scored from $w = 0$ to 4. Next, in a Monte Carlo simulation, nine independent random samples were drawn, one from each of the nine BetaPert distributions with individual estimates. Using a RiskDiscrete function in @Risk, one of these nine samples was selected, each sample weighted by the value of w per expert. By repeating this for 50000 iterations, a mean estimate and a 5% and 95% percentile estimate for each parameter was derived. For some parameters (a , b_2 , b_4 and f_{surviv}) it was assumed that the parameters were estimated on a log scale: The values of these parameters may be very small, but larger than zero. Therefore log-transformed BetaPert distributions were used for these parameters.

The resulting 'expert panel' parameter estimates are given in Table 3-17. The means of these parameter estimates are used as default values in the risk assessment. For four parameters (a , G_{prc} , W_{gb} 's) the experts were asked to assess the variability of the parameter. Here the means and the percentiles are used to derive a variability distribution of the parameter.

Table 3-16 Expert parameter estimates of 15 model parameters. The minimum, most likely and maximum estimates of the parameter values are given for nine numbered experts. The weight is a measure for the experts expertise, as indicated by him/herself. Expert nine was an expert on expert meetings, and apparently did not consider himself an expert on most topics. Experts with numbers larger than nine are experts replacing experts with weight zero. These substitutes were not present at the meeting and were asked to give a parameter estimate afterwards.

% art 4 slaughter

expert	weight	min	ml	max
1	3	0.00%	0.00%	0.01%
2	1	5%	7%	10%
3	2	2%	3%	5%
4	2	1%	2%	4%
5	1	1%	3%	10%
6	1	0.01%	0.002	0.001
7	0	0.01%	0.50%	1%
8	2	5%	10%	15%
9	0	2.50%	5%	10%

b2, clustering carcass halving

expert	weight	min	ml	max
1	3	inf.	inf.	inf.
2	2	34.31	inf.	inf.
3	1	inf.	inf.	inf.
4	2	0.05	0.05	0.05
5	1	0.20	0.64	1.61
6	2	8.31	34.31	inf.
7	1	0.05	0.64	inf.
8	3	8.31	inf.	inf.
9	0	0.15	0.64	1.61

n (industrial)

expert	weight	min	ml	max
1	1	60	80	100
2	0	40	50	80
3	0	10	30	50
4	2	25	35	50
5	0	30	40	80
6	0	0	0	0
7	0	40	50	80
8	2	25	50	75
9	0	40	70	80

m (art 10)

expert	weight	min	ml	max
1	2	1	1.2	2
2	1	1	3	5
3	3	1	4	6
4	4	2	2.5	3
5	2	1.5	2	2.5
6	1	2.5	2.8	3.5
7	2	2	5	10
8	1	2	3	4
9	0	0.5	2	4

b4 clustering trimmings

expert	weight	min	ml	max
1	3	inf	inf	inf
2	1	0.05	0.31	inf
3	0	0.18	0.18	0.18
4	3	0.05	0.66	inf
5	1	0.05	0.18	0.66
6	3	0.05	0.05	0.66
7	2	0	0.18	3.11
8	2	0.15	0.66	7000
9	0	0.18	0.66	7000

n (traditional)

expert	weight	min	ml	max
1	2	1	1	2
2	1	1	1	2
3	3	1	2	3
4	1	1	1.2	3
5	0	1	1	2
6	0	1	1	3
7	1	1	1	1.5
8	2	1	1	2
9	0	1	1.5	2

m (art 4)

expert	weight	min	ml	max
1	2	1	1.5	2
2	1	0	0.5	1.5
3	2	1	3	5
4	4	1	1	1.5
5	1	0.5	1	1.5
6	1	0.8	1	4
7	1	2	3	7
8	1	1	1	1.1
9	0	0.5	1	1.5

% tartares industrial

expert	weight	min	ml	max
1	3	0.85	0.9	0.95
2	1	0.5	0.75	0.85
3	1	0.8	0.8	0.8
4	0.5	0.2	0.3	0.5
5	1	0.5	0.6	0.8
6	1	0.3	0.4	0.7
8	2	0.85	0.9	0.95
9	0	0.75	0.85	0.9
20	5	0.78	0.79	0.8

f wd

expert	weight	min	ml	max
1	3	10.0%	20.0%	30.0%
2	2	3.0%	5.0%	15.0%
3	0	2.0%	5.0%	20.0%
4	1	10.0%	25.0%	35.0%
5	1	10.0%	20.0%	25.0%
6	2	20.0%	25.0%	50.0%
7	2	0.5%	3.0%	5.0%
8	2	20.0%	33.0%	50.0%
9	1	25.0%	40.0%	60.0%

a

expert	weight	min	ml	max
1	2	0.1	1	3
2	2	0	0.7	2
3	2	2	2	2
4	2.5	0	1	5
5	1.5	0	1	5
6	2	0	0.5	3
7	1	1	2	3
8	2	1	5	10
9	0	0.1	0.5	4

Wg (industrial)

expert	weight	min	ml	max
1	1	1	20	100
2	0	5	15	40
5	0	750	1500	2000
6	1	20	25	100
8	1	500	1000	1500
9	0	500	1000	1500
10	5	180	190	200
11	0.5	1000	1500	2000
12	1	500	750	1000

f surv med

expert	weight	min	ml	max
1	3	0.1	0.2	0.3
2	2	0.1	0.2	0.3
3	0	0.2	0.5	0.8
4	1	0.6	0.7	0.8
5	1.5	0.1	0.25	0.5
6	2	0.1	0.1	0.2
7	3	0.5	0.7	0.8
8	1	0.15	0.2	0.25
9	0	0.15	0.2	0.3

Gprc

expert	weight	min	ml	max
1	3	0.1	0.666667	1
2	2.5	0.5	1	2
3	0	1	1	1
4	1	1	2.5	4
5	2	0.5	1	10
6	3	0.666667	1	1.5
7	3	0.5	1	2
8	1	0.001	0.1	2
9	0	0.05	0.2	2

Wg (traditional)

expert	weight	min	ml	max
1	1	0.5	2	5
2	0	1	5	10
3	0.1	1	3	5
6	0.5	3	5	10
8	3	1	2.5	5
10	4	2	10	20
13	3	10	12	15
14	3	0.5	10	25
15	2	1	2	4

f surv wd

expert	weight	min	ml	max
1	3	0.001	0.01	0.02
2	2	0	0.0001	0.0005
3	0	0.1	0.2	0.5
4	1	0.01	0.1	0.15
5	1.5	0	0.0001	0.01
6	2	0	0.0001	0.0001
7	3	0	0.00001	0.0001
8	1	0.000001	0.0001	0.001
9	0	0.005	0.01	0.02

Table 3-17 Results of 50000 iterations of the workshop results. Mean values and 5% and 95% percentiles of the RiskDiscrete functions that combine the individual expert estimates are given for 15 model parameters. For four parameters (a , G_{prc} , W_{gb} 's) the distributions indicate variability, for the others uncertainty. inf= infinite

OVERVIEW	5%	mean	95%
% art. 4 slaughter	0.00%	3.48%	11.11%
% tartares industrial	38.45%	77.12%	92.13%
m (art. 10)	1.23	2.98	5.73
m (art. 4)	0.66	1.61	3.82
a (gram faeces per carcass)	0.00	0.31	5.08
G_{prc}	-2.32	-0.30	1.25
b_2	0.00	2.7	inf.
b_4	0.16	0.73	inf.
W_{gb} (industrial)	22.67	370.12	1300.70
W_{gb} (traditional)	1.60	7.72	15.43
n (industrial)	30.07	50.33	85.54
n (traditional)	1.01	1.44	2.40
f_{wd}	2.56%	20.49%	41.19%
$f_{\text{surviv, medium}}$	10.54%	26.48%	73.78%
$f_{\text{surviv, well done}}$	0.00%	0.01%	6.24%

3.3.4.2 The food pathway

Step 1: Contamination of carcasses

parameters:

P_f , C_{ij} , n_{mean} , m_{mean} , a_{mean} , a_{sd} , b_1

Although much research concentrates at the occurrence of STEC O157 in cattle at the farm and prior to slaughter, there is little data on the (yearly) mean prevalence at animal level, for animals at slaughter. Based on literature data (see 3.2.1) the prevalence P_f is estimated at 0.01. This prevalence is close to the mean of the Beta (2.7, 250) distribution used by Cassin et al. (1998)

The concentration of STEC O157 in faeces at the moment of slaughter (C_{ij} , the concentration in animal j 's faeces, contaminating carcass i) is an important parameter. However, data are scarce. We found no data for Dutch cattle, and no other reference, than that applied by Cassin et al. (1998). We therefore use the same (North American) data of Zhao et al. (1995): the variability of the concentration is implemented in @Risk as a Cumulative distribution (Vose 2000): $\log C \sim \text{RiskCumul}(0,6, (2,3,4,5), (0.46875, 0.53125, 0.875, 0.96875))$. This distribution is illustrated in Fig 3-9.

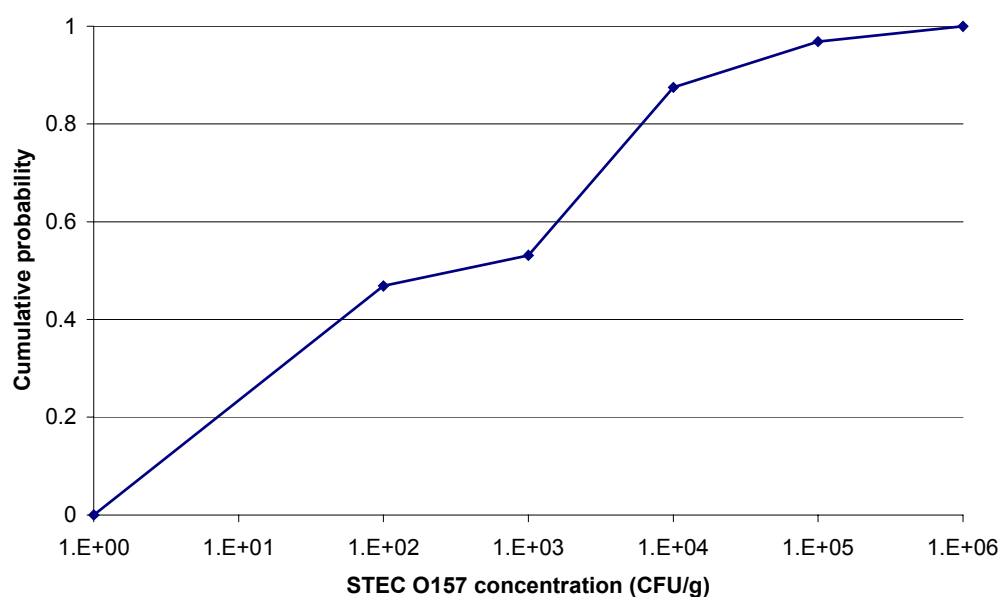


Figure 3-9 The variability in the concentration of STEC O157 in cattle faeces (C_{ij}) as implemented in the risk model. Data of Zhao et al. (1995) are used to construct a Risk Cumul probability distribution function.

The mean number of carcasses used for a ground beef batch (n_{mean}), and the mean number of animals from which the faeces contaminates a single carcass (m_{mean}) are assessed by the expert panel for different types of slaughterers and butchers. The resulting variability distributions are given in Figure 3-10.

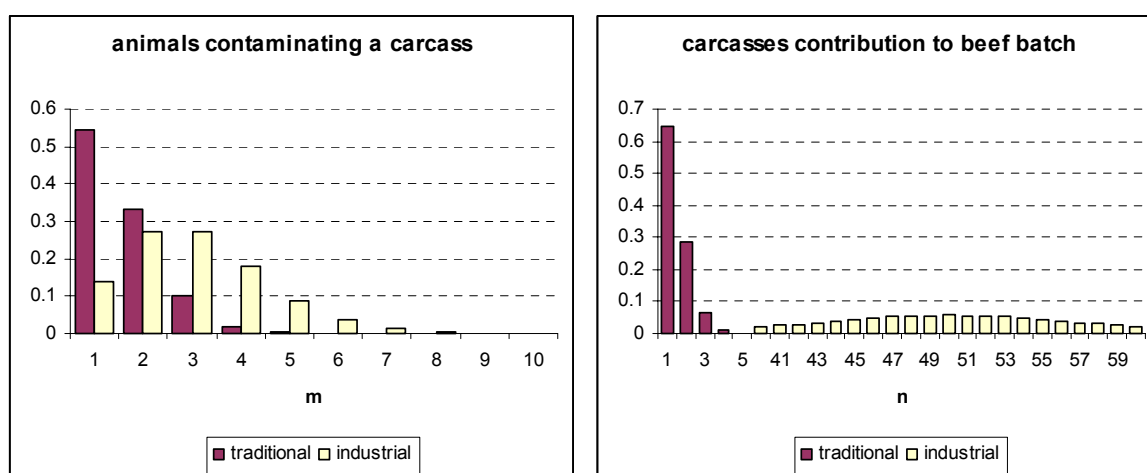


Figure 3-10 Variability in the number of animals contaminating a carcass (m) and variability in the number of carcasses contributing to the ground beef batch (n) as assumed in the model. For ‘traditional’ slaughter (art. 4) $m_{\text{mean}} = 1.61$, for ‘industrial’ slaughter (art 10.) $m_{\text{mean}} = 2.98$, for the ‘traditional’ butcher $n_{\text{mean}} = 1.44$, for the ‘industrial’ butcher $n_{\text{mean}} = 50.33$ in the baseline model.

In the risk assessment model we use a parameter a indicating the contamination of cattle carcasses with faeces, in gram faeces per carcass. Our aim is to use this parameter to convert

concentrations in faeces (which are usually expressed as(log) cfu/g), to carcass contamination (in cfu/carcass). If the latter is explicitly expressed as whole numbers per carcass, one prevents using concentrations between zero and one cfu per carcass.

Unfortunately we have no direct data available on the grams of faeces per carcass in the Netherlands. We therefore asked the expert panel to assess carcass contamination, by estimating minimum, most likely and maximum values for a . The logs of these estimates were used to derive the expert panel probability distribution of a , as explained in 3.3.4.1. This distribution, which expresses the variability in carcass contamination, was fitted to several well known probability distribution using the 'BestFit' program that is part from @Risk 4.0 (Palisade). The first option was to fit the logs of the expert estimates to a normal distribution, so that the variability of a would be given by a lognormal distribution (as in Cassin et al. (1998), see below). However, as illustrated in Fig 3-10, it appeared that a 'Beta general' function (Vose 2000) fitted better to the expert distribution. This was particularly relevant because the 'BetaGeneral' has a maximum value (a_{\max}), whereas a normal distribution has not. This tail of large quantities of faeces on the carcass may be unrealistic (with more than 100 g faeces per carcass) and may have a large impact on the risk. Therefore the variability in carcass contamination was described by a BetaGeneral($\alpha, \beta, 0, a_{\max}$) distribution, which is identical to a $a_{\max} \times \text{Beta}(\alpha, \beta)$ distribution. The best fitting values were $\alpha = 0.395$, $\beta = 2.473$ and $a_{\max} = 10.1$ g.

The results of this expert elicitation can be compared to (North American) literature data. Cassin et al. (1998), who used a different approach than the one we use, converted the concentration in faeces to g per cm² carcass by a dilution factor F_{DIL} expressed as g/cm². This describes the concentration on the carcass after dehiding. They use data from *E. coli* Biotype I in bovine faeces (unpublished Australian data from 500 samples) and USDA (1994) baseline data on beef carcass surfaces (>2000 samples). From these data they derive a Normal (-5.1, 0.9) [log g/cm²] distribution for F_{DIL} . The decontamination following dehiding has an effect which Cassin et al. (1998) describe by a factor R_{DEC} which has a Uniform(1,2.5) [log cfu/cm²] distribution. These process values can be implemented in our model. For a carcass area of 4m², the relation between a and Cassin's parameters is $a = 4 \cdot 10^4 \cdot 10^{F_{\text{dil}}[-R_{\text{dec}}]}$ g/carcass. Here the parameter R_{DEC} , between [], is optional, as the decontamination step can not be identified as such in Dutch slaughterhouses. It is unclear whether it should be included when describing Dutch slaughterhouse practices.

The resulting characteristics of a , using the distributions of Cassin et al. (1998), are given in Table 3-18 and Figure 3-11 and compared with the expert estimates and the fitted distribution used in our study. It illustrates that the Cassin model, omitting R_{DEC} , fits fairly well to the experts estimates, and that a Beta distribution fits better than a (log-)normal distribution.

Cassin et al. (1998) use data on faeces contamination and carcass contamination of *E. coli* Biotype I to derive their transition factors. We found other, similar data in the literature (Diez-Gonzalez et al. 1998, Jordan and McEwen 1998, Midgley et al. 1999, Bacon et al. 2000, Calicioglu et al. 1999). It shows that *E. coli* (biotype I) concentrations in cattle faeces are strongly influenced by the diet of the animals, and that carcass chilling may give a decrease of *E. coli* concentrations present. Using data of Midgley et al. (1999), (mean 6 log

cfu per g faeces), and Bacon et al. (2000) (mean about 4 log cfu per 100 cm² on the carcass after dehiding and 2 log cfu per 100 cm² after evisceration and decontamination), the geometric mean of carcass contamination can be estimated at 4 or 0.04 g faeces per carcass, excluding and including decontamination. These values are somewhat larger than those applied in this study.

Table 3-18 Characteristics of the distribution of parameter a , indicating the level of faecal contamination of cattle carcasses. Values are given for the experts estimates (Experts), the fitted Beta distribution (Beta-model), and the distributions of a as applied by Cassin et al. (1998), both excluding (F_{DIL}) and including the effect of decontamination ($F_{DIL-R_{DEC}}$). μ is the mean (g/carcass), sd is the standard deviation, μ log is mean of the logs (log g/carcass), sd log its standard deviation, and $geo \mu$ the geometric mean (g/carcass). For illustration see Fig 3-11.

	Experts	Beta-model	F_{DIL}	$F_{DIL-R_{DEC}}$
μ	1.41	1.39	2.68	0.075
sd	1.67	1.77	16.34	0.552
μ log	-0.50	-0.50	-0.50	-2.248
sd log	1.18	1.15	0.90	1.005
$geo \mu$	0.32	0.32	0.32	0.0057

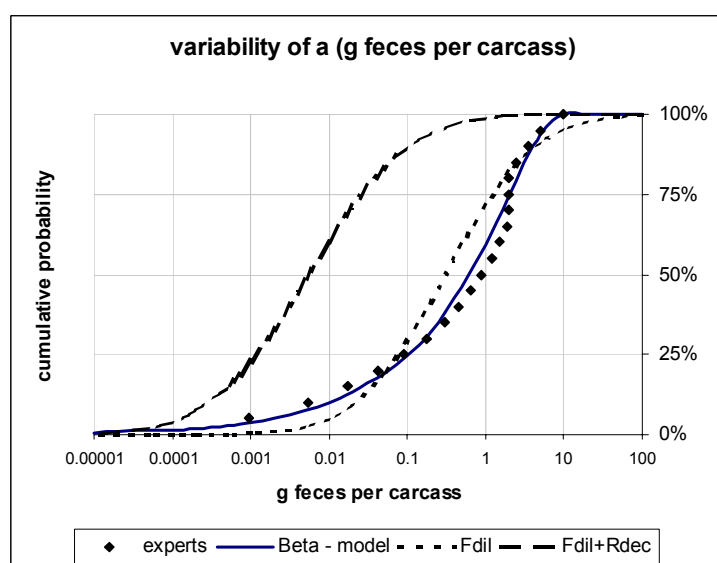


Figure 3-11 The variability in carcass contamination a , expressed in gram faeces per carcass. The distribution derived from the experts estimates (experts) and the fitted Beta distribution (Beta-model) are given, and compared with the (log normal) probability distributions of a , excluding and including the effect of decontamination, derived from Cassin et al. (1998). (See table 3-18 and main text.) Note the frequent relatively low values of a (smaller than 1 g per carcass) and the impact of decontamination. Also note the bad fit of the log normal distribution to the expert-distribution at the right hand tail of the distribution.

The beta factor b_1 , as a measure for the relative contribution of animals in the faecal contamination of the carcass, was unknown and set at 1 by the authors. (For explanation and illustration see Appendix 3.)

Step 2: Partitioning to half carcasses

parameters:

W_{carc} b_2

According to PVE (2001) mean carcass weight at slaughter of cows, heifers and steers in 1997 was 320 kg. This is incorporated in the model as a fixed number: $W_{\text{carc}} = 320$ kg. The clustering of STEC O157 at carcass halving (b_2) is assessed by the expert panel as indicated in Table 3-17. The mean estimated value of b_2 is 2.7, which on average stands for a distribution as illustrated in Figure 3-12 with 67% of the cells on the half carcass with most cells.

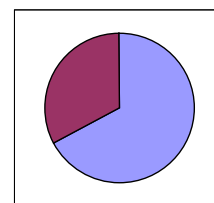


Figure 3-12 Clustering at carcass halving

Step 3: Growth and inactivation on the carcasses

parameters:

G_{prc}

The variability in growth or inactivation of STEC O157 on the carcass, expressed in the number of cell doublings (or halvings), G_{prc} , (see Cassin et al. 1998), is assessed by the expert panel as indicated in Table 3-17.

Step 4: Partitioning to trimmings

parameters:

b_4

The clustering of STEC O157 at trimming (b_4) is assessed by the expert panel as indicated in Table 3-16. The mean estimated value of b_2 is 0.73, which (for example for $k=6$) on average stands for a distribution as illustrated in Figure 3-13.

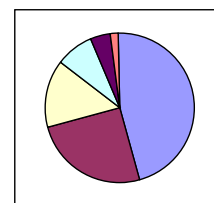


Figure 3-13 Clustering in trimmings

Step 5: Mixing: the ground beef batch

parameters:

W_{gb} b_5 r_{corr}

The weight of the ground beef batch (W_{gb}) is highly variable. Different experts on this topic gave different estimates, both for traditional and industrial produced ground beef (destined to become tartare), probably because different experts are familiar with different slaughtering practices. Based on all this information, the variability in ground beef batches is modelled with a BetaPert distribution, with a minimum, most likely and maximum value for traditional and industrial ground beef batches, as indicated in Table 3-21.

The beta factor b_5 , as a measure for the relative contribution of (trimming of the) carcasses in the ground beef batch, is set as $b_5 = 1$ by the authors. (For explanation and illustration see Appendix 3.)

As the weight of the ground beef batch is probably correlated to the number of animals contributing to the batch, a correlation r_{corr} between n and W_{gb} is implemented in the @Risk model. As default we assumed that $r_{corr} = 0.8$.

Step 6: Partitioning to tartare patties

parameters:

b_6 , W_{tt} for 3 age classes

The beta factor b_6 , as a measure for the level of clustering of cells when tartare patties are made, is assessed by the authors. As the clustering effect may be rather large due to the fact that large batches are not easily mixed well, as default it is assumed that $b_6 = 0.15$, on average representing a distribution as given in Figure 3-14 (with $k = 6$ and $N = 100$).

The third Dutch nutrition surveillance (Kistemaker et al. 1998) gives detailed information of everything that 6250 Dutch people consumed during two consecutive days. It gives information on the weight of the tartare portions consumed in different age classes. Table 3-19 lists the mean value, minimum, maximum, standard deviation and number of consumptions per age class. The distributions of the weight appeared to fit quite well to log normal distributions, with mean and standard deviation as given. Therefore the distribution of tartare weights (W_{tt}) per age class are assumed to be variable according to lognormal distributions thus characterised.

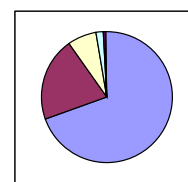


Figure 3-14
Clustering in steak tartare patties

Table 3-19 The weights of the steak tartare portions consumed in the Netherlands, as derived from the Kistemaker et al. (1998) data. Mean minimum, maximum and standard deviation and sample size of the data are given, with mean and standard deviation of the best fitting lognormal distribution used in the risk model (Lnorm mean and Lnorm sd).

	1-4	5-14	15+	total
mean	53.16	82.52	98.12	92.78
min	10	28	8	8
max	100	133	400	400
sd	26.80	19.74	42.78	41.85
n	19	23	185	227
Lnorm mean	54.1	83	99	94
Lnorm sd	32.4	24.1	45.3	46.9

Step 7: Growth of E coli during tartare storage

parameters:

$T_{\text{mean}}, T_{\text{sd}}, t_{\text{mean}}$

There were no data available on storage time and storage temperature of steak tartare in the Netherlands. Therefore we applied a distribution of mean temperatures in domestic refrigerators close to one used in a previous risk assessment (Nauta 2001b). For storage time we apply a exponential distribution with a mean storage time of 24 h, derived from the fact that the ‘Voorlichtingsburo Vlees’ states that ground meat should be kept one day in the refrigerator below 5°C at maximum. This means that it is assumed that 27% of the consumers don't keep to the advised storage time.

$$T \sim N(7,2) \text{ } ^\circ\text{C},$$

$$t \sim \text{Exp}(24) \text{ h}$$

With the model given by (3.6) and (3.7) , at $T = 7^\circ\text{C}$, $\text{LPD} = 123 \text{ h}$ and $\text{GT} = 435 \text{ h}$. This implies that the storage time should exceed five days before the onset of growth. Presumably growth of STEC O157 in tartare is not very important. Extended effort in determining precise growth modelling and storage conditions therefore has no priority.

Step 8: Inactivation during preparation of patties

parameters:

$f_{\text{surviv, pst}}, f_{\text{wd}}$

In the expert workshop the experts were informed with some of the hamburger STEC O157 inactivation information. (For ‘medium raw’ hamburger $f_{\text{surv}} \approx 0.01$, and for well done hamburgers $f_{\text{surv}} \approx 10^{-5}$) They were then asked to assess the survival of *E. coli* O157 in tartare patties that are kept ‘medium raw’ and that are cooked ‘well done’. f_{wd} is discussed below (step 9).

Step 9: Exposure at consumption

parameters:

$P_{\text{cons, ac, pst}}, I_{\text{ac}}$

To assess the exposure to STEC O157 at population level, consumption data are required. For this purpose the information on consumption of tartare from the third Dutch nutrition surveillance (Kistemaker et al. 1998) is used. As mentioned for Step 6, this surveillance study gives detailed information of everything that 6250 Dutch people consumed during two consecutive days. Unfortunately, however, it does not provide all details relevant for microbiological risk assessment. The consumption data on ‘tartare’ does make a distinction between categories ‘raw’ and ‘prepared’, but not according to a definition practical for microbiological risk assessment. ‘Raw’ need not necessarily mean ‘raw’ in the sense of ‘non cooked’ and if ‘prepared’, there is no clue about heat treatment. Therefore it is difficult to assess the relative frequencies of different preparation styles on the basis of the Kistemaker et al. (1998).

Table 3-20 below gives the Kistemaker et al. (1998) data and the calculated values of the (expected) probabilities of consumption of tartare P_{cons} for the categories ‘raw’ and ‘prepared’, of a person in the age class, on a given day. For the distinction between ‘raw’ and ‘prepared’ the official definition of Kistemaker et al. (1998) is used, completed with some additional unpublished information from one of the authors. We had no other information to assess consumption frequencies.

The probabilities given next to the heading ‘Classic’ are calculated by classical statistics, as x/n , the means from the data. As in the younger age classes data are limited, with little tartare consumption and no ‘raw’ tartare consumption at all, the estimated probability of raw tartare consumption (0%) is probably too low, as a consequence of the (relatively) small sample size. As an alternative we used Bayesian statistics. Here the probabilities are calculated using the Beta distribution with Jeffrey’s prior (Beta(0.5,0.5)). This gives larger expected values for the tartare consumption, especially in the youngest age classes. In the 1-4 age class the estimated prevalence of raw tartare consumption is higher than in the 15+ age class, although no raw tartare consumption was found in the 1-4 age class. This is a consequence of the (subjective) choice of the prior. When the ‘traditional’ uninformed prior, the uniform (Beta (1,1)) is chosen (Vose 2000), this effect is even worse: It renders unrealistically high estimates of raw tartare consumption in the youngest age classes.

Alternative priors Beta (0.06,0.94) and Beta(0.12,1.88), based on the information of the oldest age class, give results similar to Jeffrey's prior. The latter is chosen because it suggests 'data independence'.

Table 3-20 The Kistemaker et al. (1998) data on the consumption of steak tartare in three age classes in the Netherlands. First the 'numbers consumed' are given. In two days 6250 people ate 227 tartare portions, giving an estimated mean probability of consumption of 1.82% per person per day. The mean probabilities of consumption per age class and in the categories 'raw' and 'prepared' are calculated using both 'classical' and Bayesian statistics, as explained in the main text.

		total	1-4	5-14	15+	
Data:	numbers					
	consumed	raw	6	0	0	6
		prepared	221	19	23	179
	<i>n</i>	6250	347	831	5072	
Classical		raw	0.05%	0.00%	0.00%	0.06%
		prepared	1.77%	2.74%	1.38%	1.76%
		total	1.82%	2.74%	1.38%	1.82%
Bayesian		raw	0.05%	0.07%	0.03%	0.06%
		prepared	1.77%	2.81%	1.41%	1.77%
		total	1.82%	2.88%	1.44%	1.83%

The prepared tartare patties are separated in two classes 'medium' and 'well done', indicating a different level of inactivation. There was no data on consumer behaviour available to quantify the relative frequency in which each preparation style is applied in the Dutch population. Therefore the percentage of tartare patties thoroughly heated ('well done'), f_{wd} , is assessed by the expert panel as indicated in Table 3-21.

For the number of inhabitants in NL per age class data from 1998 of 'Statistics Netherlands' were used (CBS 2000).

Table 3-21 Overview of the default values (with data sources) of the baseline model parameters

P_f	0.01	(section 3.2)
C_{ij}	Zhao distribution	(Cassin et al. 1998, Zhao et al. 1995)
n_{mean}	1.44 / 50.33	(experts, traditional / industry)
m_{mean}	1.61 / 2.98	(experts, traditional / industry)
a_{max}	10.1 g/carcass	(experts)
α	0.395	
β	2.473	
b_1	1	(author guess)
b_5	1	(author guess)
W_{carc}	320 kg	(PVE 2001)
G_{prc} (min, ml, max)	< -2.3, -0.3, 1.25 >	(experts)
W_{gb} (min, ml, max) (kg)	< 1.6, 7.7, 15.4 > < 22.7, 370, 1300 >	(experts, trad.) (experts, industry)
W_{tt} (mean, sd) (g)	fitted to Lognormal distribution, Kistemaker et al. (1998) data	
	age class	1-4 5-14 15+
	mean	54.1 83 99
	sd	32.4 24.1 45.3
b_2	4.02	(experts)
b_4	1.06	(experts)
b_6	0.22	(author guess)
$T_{\text{mean}}, T_{\text{sd}}$	7°C, 2°C	(Nauta 2001b)
t_{mean}	24 h	(Vleesinfo 2001)

f_{surviv} raw 100%, (experts based on Jackson et al. 1996)
 med 26%,
 well done 0.01%

f_{wd} 20.4% (experts)

$P_{\text{cons, ac, pst}}$

Probability of consumption of a tartare patty prepared as indicated, per person from each age class per day

	1-4	5-14	15+	total
raw	0.07%	0.03%	0.06%	0.05%
med	2.23%	1.12%	1.41%	1.42%
well done	0.57%	0.29%	0.36%	0.36%
total	2.88%	1.44%	1.83%	1.84%

(Kistemaker et al. 1998)

I_{ac}

780960, 1939700 and 1284440 per age class resp.
 (CBS 2000)

DR parameters

(Chapter 4)

	1-14	15+
r	0.0093	0.0051
a	0.102	0.0667
b	2.34	3.02

relative production per exposure route

(experts)

industry art 10: 77.1%
 traditional art 10 19.4%
 art 4: 3.5%

r_{corr} correlation n and W_{gb}

0.9

(author guess)

3.3.5 The spreadsheet model

The model outlined above has been implemented as a spreadsheet model in @Risk 4.0.5, an add on on Excel, running on a PC. The program uses Monte Carlo sampling to simulate the transmission of STEC O157 along the food pathway. In the model, the ground beef batch is the central unit. As illustrated in Figure 3-6 a ground beef batch is formed from trimmings from n carcasses (with index i , $i=1..n$), that are contaminated with faeces from m_i animals. Next, the ground beef batch is used for the production of ten tartare patties, which are independently modelled as patties prepared in three preparation styles, for people from three age classes, 1-4, 5-14 and 15+ (15 and older), that is as $3 \times 3 = 9$ independent tartare patties with nine different destinations.

For each of the three exposure routes (see Figure 3-7) a set of 50000 independent iterations of the model was run up to Model Step 8. By adjusting for the relative contribution for each exposure route (industry art 10: 77.1%; traditional art 10 19.4%; art 4: 3.5%), the prevalence of contaminated tartare patties in the Netherlands and the variability distribution of numbers of STEC O157 in raw tartares in the Netherlands can be derived. These were used to calculate exposure after preparation. By linking this exposure with the effect model (chapter 4), the number of cases resulting from STEC O157 as a consequence of steak tartare consumption can be predicted with the model. For this purpose a Monte Carlo of 25000 iterations with raw tartare patties positive for STEC O157 was run in @Risk.

In one run of the Monte Carlo simulations, parameter values are sampled from probability distributions as described above, all representing variability. As discussed elsewhere, uncertainty has not been incorporated in this study for several reasons.

4. Effect model

4.1 Introduction

Escherichia coli O157 dose response relationships have previously been investigated based on experimental infection in rabbits (Haas et al. 2000) and based on surrogate pathogens enteropathogenic *E. coli* and *Shigella dysenteriae* (Powell et al. 2000). Neither of these studies, however, are based on *E. coli* O157 infection in humans, simply because appropriate data were not available.

In September 1996, a Shiga toxin producing *E. coli* (STEC) O157:H7 outbreak occurred in an elementary school in Morioka city, Japan (Shinagawa 1997). Children and teachers ate a school lunch prepared by a catering service. The setting in which the outbreak occurred was unique. First, the primary school represents a well-defined community at the time of exposure. Consequently, the total number of exposed people is accurately known. Furthermore, it was possible to collect faecal samples of all those who had consumed the school lunch. Second, catering services preserved a meal for two weeks period subsequent to the preparation. Availability of the suspected food sample enabled the local health authority to run a quantitative assay. Third, the average consumption per child could reliably be estimated, due to uniform preparation of the lunch and the practice among children to completely eat what is provided. Combining this information, it is now possible to relate the number of STEC O157:H7 ingested by a child and the likelihood of infection, indicated by excretion.

4.2 Materials and methods

4.2.1 Outbreak data

The STEC O157:H7 outbreak has previously been reported in Japanese (Shinagawa 1997). Ten days following the suspected day of consumption, stool samples were collected from the total 842 children (440 boys and 388 girls, 828 ate the school lunch) and 43 teachers. Stool samples of 208 children and 7 adults were tested positive for STEC O157:H7 (Table 1). Positive stool samples were seen in comparable frequencies across age and sex, suggesting that the level of contamination was roughly the same across all school lunches.

Following examination of the school lunch, STEC O157:H7 were isolated from salad and seafood sauce by a magnetised beads based immunoassay. The concentration of pathogens was determined by MPN method. Estimates ranged between 4 to 18 cfu per 100 gram. Taking the arithmetic mean, we estimate the average dose to be 11 cfu per 100 gram.

Table 4-1 STEC O157:H7 outbreak in Japanese elementary school

	Exposed <i>n</i>	Faecal negatives <i>k</i>	Average ingested dose <i>D</i>
Children	828	620	31 cfu person ⁻¹
Teachers	43	36	35 cfu person ⁻¹

The salad and the seafood sauce were provided to the children in amounts according to their age. Amounts varied from 225 gram to 320 gram. The average amount of salad and seafood sauce was 280 gram. Age-dependent doses due to different amounts of consumption had minimal effect on the outcome of the subsequent analysis. For this reason, we estimate the dose based on the average consumption. Because it is likely that most children completely ate the school lunch, a child ingested on average 31 cfu of STEC O157:H7. The teachers consumed as much as the children of the senior class did, i.e. 320 gram. Having consumed slightly more, the teachers ingested on average 35 cfu per person.

STEC O157:H7 were not detected from ingredients of the salad and the seafood sauce. This finding suggests that the pathogen had accidentally been transported to the catering service after the meal had been prepared. However, the exact route of contamination was not identified.

4.2.2 Binomial likelihood

Following a consumption of the school lunch, a child either becomes infected or remains unaffected by the pathogens. We denote by the symbol p the probability that a child remains free of the pathogen. Having examined the total n faecal samples and found k of those to be free of STEC O157:H7, the binomial likelihood is,

$$l(n, k, p) = (1 - p)^{n-k} p^k \quad (4.1)$$

Intuitively, the best estimate for the parameter p is, $p = k/n$, which is also the maximum likelihood estimate.

4.2.3 Exponential model for the parameter p

To extrapolate the probability of infection to doses other than the dose estimated from the outbreak, we derive exponential model for the parameter p . The exponential model has been used in analyses of water and food borne diseases (Haas 1983, Peto 1953, Teunis et al. 1996). An ingested pathogen travels a long way from the mouth to the intestine, surviving a series of host defence barriers and ultimately colonising the intestinal tract. Many others however, will perish along the journey. We derive a model by first considering that the probability of

infection by one pathogen is equal to a non-zero value r . Furthermore, we assume that pathogens act independently to infect a host. By virtue of the independent action, the probability is equal to $(1-r)^j$ that all j micro-organisms ingested fail to infect the host.

The batch of the salad and seafood sauce is assumed mixed well during the preparation process. In the homogeneous batch, the number of the pathogens is Poisson distributed with the average concentration equal to the MPN. Multiplying the MPN by the amount of consumption, we calculate the average ingested dose per person ($=D$). The actually ingested number of the pathogen is equal to j with the probability,

$$\frac{e^{-D} D^j}{j!} \quad (4.2)$$

A host who consumed the contaminated food would remain uninfected with the probability p ,

$$p = \sum_{j=0}^{\infty} (1-r)^j \frac{e^{-D} D^j}{j!} = e^{-rD} \quad (4.3)$$

This is the exponential model. Since $p = k/n$, the maximum likelihood estimate for the parameter r must satisfy the relationship,

$$e^{-rD} = \frac{k}{n} \quad (4.4)$$

Upon inversion, we obtain the maximum likelihood estimate for the parameter r

$$r = \frac{1}{D} \ln\left(\frac{n}{k}\right) \quad (4.5)$$

4.2.4 Hypergeometric model for the parameter p

Contrary to the assumption that the probability r of infection per micro-organism is constant, virulence of the pathogens and susceptibility of children may considerably differ in a realistic situation. To take such heterogeneity into account, the parameter r may be assumed to follow the beta distribution. The probability density function is,

$$\frac{r^{a-1} (1-r)^{b-1}}{\text{Beta}(a,b)} \quad (4.6)$$

Assuming the beta distribution for the parameter r leads to the hypergeometric dose response model (Teunis and Havelaar 2000).

$$p = \int_0^1 e^{-rD} \frac{r^{a-1} (1-r)^{b-1}}{\text{Beta}(a,b)} dr \quad (4.7)$$

The integral defines the confluent hypergeometric function (Teunis and Havelaar 2000),

$${}_1F_1(a, a+b, -D) \quad (4.8)$$

The hypergeometric model may be approximated by the Beta-Poisson model,

$${}_1F_1(a, a+b, -D) \approx \left(1 + \frac{D}{b}\right)^{-a} \quad (4.9)$$

if the conditions $1 \ll b$ and $a \ll b$ are satisfied (Teunis and Havelaar 2000).

The parameter values are estimated by means of Markov Chain Monte Carlo (MCMC) algorithm, which we adopted from (Gilks et al. 1996) and implemented in Mathematica (Wolfram 1999).

For computational purpose, it is convenient to transform the parameters by $(a, b) = (10^\alpha, 10^\beta)$. Substituting the hypergeometric model (Eq.4.8) into Eq.4.1, we obtain the binomial likelihood,

$$l(\alpha, \beta, D, n, k) = (1 - {}_1F_1(10^\alpha, 10^\alpha + 10^\beta, -D))^{n-k} {}_1F_1(10^\alpha, 10^\alpha + 10^\beta, -D)^k \quad (4.10)$$

Prior distribution for the new parameters α and β is taken to be a normal distribution,

$$g(\alpha, \beta, \mu_\alpha, \mu_\beta, \sigma_\alpha, \sigma_\beta) = \frac{1}{2\pi\sigma_\alpha\sigma_\beta} e^{-\frac{(\alpha-\mu_\alpha)^2}{2\sigma_\alpha^2} - \frac{(\beta-\mu_\beta)^2}{2\sigma_\beta^2}} \quad (4.11)$$

The posterior distribution is proportional to the product of the binomial likelihood and the prior distribution.

$$l(\alpha, \beta, D, n, k) \times g(\alpha, \beta, \mu_\alpha, \mu_\beta, \sigma_\alpha, \sigma_\beta) \quad (4.12)$$

The MCMC produces samples from the posterior distribution, which describe how uncertain the values for the parameters are. The median of the posterior samples approximates the most likely set of values for the parameters.

4.3 Results

Based on the exponential model, the probability of infection, i.e. faecal positive, per cfu of ingested micro-organism is approximately 0.01 for the children (Table 2). Equivalently, if every 100 children ingested exactly one STEC O157:H7, on average one child would become infected. The probability of infection for the teachers is estimated to be half of that for the children. The difference is not statistically significant, because a relatively small number of teachers experienced the outbreak. Despite this, the trend that a child is more susceptible to the pathogen than an adult seems reasonable.

As opposed to a constant probability of infection, the hypergeometric model allows the probability to vary. Individual-specific susceptibility and pathogen-specific virulence are both important reasons why the probability of infection is not constant. However, with the data at

hand, variation in infectivity among pathogens cannot be separated from variation in susceptibility to infection among human hosts. We will speak of varying probability of infection.

Table 4-2 Parameter estimates in this report and those as reported by other authors.

Hosts	Pathogens	Refs	Exponential	Hypergeometric		Beta-Poisson	
			e^{-rD}	${}_1F_1(a, a+b, -D)$		$\left(1 + \frac{D}{b}\right)^{-a}$	
			r	a	b	a	b
Children	STEC O157	*	$9.3 \times 10^{-3} \text{ cfu}^{-1}$	0.1	2.3	NA	NA
Adults	STEC O157	*	$5.1 \times 10^{-3} \text{ cfu}^{-1}$	0.07	3.0	-	-
Rabbit	STEC O157	Haas et al. 2000	$6.3 \times 10^{-8} \text{ cfu}^{-1}$	-	-	0.49	1.9×10^5
Humans	EPEC and <i>S. dysenteriae</i>	Powell et al. 2000	-	-	-	0.22	8722

(*) This report. (-) not reported.

The beta distribution with the estimated parameter values of the hypergeometric model (Table 2) may be summarised by quartiles. For the children, quartiles are ($2.80 \times 10^{-7} \text{ cfu}^{-1}$, $2.87 \times 10^{-4} \text{ cfu}^{-1}$, $1.69 \times 10^{-2} \text{ cfu}^{-1}$) = (25 percentile, the median, 75 percentile). For the teachers, quartiles are ($5.83 \times 10^{-10} \text{ cfu}^{-1}$, $1.17 \times 10^{-5} \text{ cfu}^{-1}$, $3.85 \times 10^{-3} \text{ cfu}^{-1}$). Compared to the estimate based on the exponential model, more than 25% of the children are more susceptible to STEC O157 ($1.69 \times 10^{-2} \text{ cfu} > 9.3 \times 10^{-3} \text{ cfu}$).

Our estimates indicate that STEC O157 is highly infectious (Table 2). This is in contrast to the previous studies (Fig.1), which are based on experimental O157 infection in rabbits (Haas et al. 2000), or the use of surrogate pathogens (EPEC and *Shigella dysenteriae*) in human infection (Powell et al. 2000). A reason for this discrepancy is that STEC O157 is much less infectious in rabbits than in humans. Furthermore, EPEC is much less infectious than STEC O157 in humans. Interestingly, the Japanese O157 outbreak data indicate the probability of infection comparable to *Shigella dysenteriae*, one of the two surrogate pathogens used in the study by Powell et al. (2000).

The models and the parameter values listed in Table 2 are visualised. HyG: Hypergeometric model (only that of children is shown), Exp: Exponential model, BP: Beta Poisson model. The models of (Haas et al. 2000) and (Powell et al. 2000) clearly do not fit to the outbreak data (open circle).

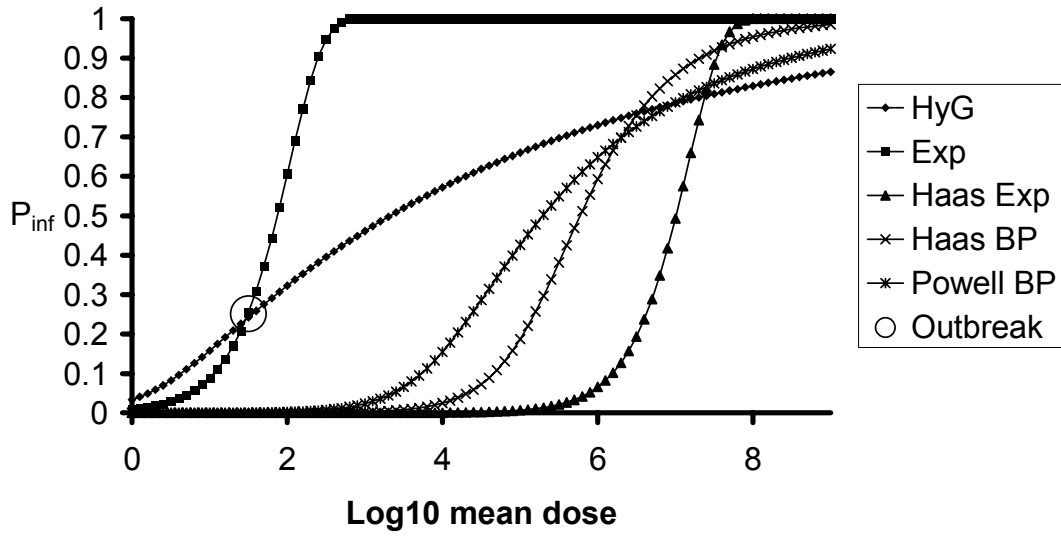


Figure 4-1 Overview of the STEC O157 dose response models

5. Risk characterisation

5.1 Results of the baseline model

The risk assessment model, combining the exposure and effect model, was run with default parameter values as given in sections 3.3.4. and 4.3, using a spreadsheet computer model, as explained in section 3.3.5.

As noted in section 3.3.3. and illustrated in Figure 3-7, the food pathway is split up in three different routes of exposure (article 10 slaughter with ‘industrial’ butcher, article 10 slaughter with ‘traditional’ butcher and article 4 slaughter). The differences between these three routes in terms of model characteristics are given in Table 5-1. For these three routes, the prevalence and the mean number of cfu per positive unit is evaluated at two points in the food pathway: the ground beef batch and raw steak tartare patties. The results are given in Tables 5-2 and 5-3.

For industrial ground beef batches (art. 10 ind), the prevalence is highest. These large batches contain meat of more animals, and thus have a larger probability that one or more contributing carcasses contain STEC O157. The mean number of cfu per positive batch is highest for the traditional ‘art. 4’ route. If such a batch is positive, it contains meat of only one or a few carcasses, and STEC O157 contamination is not diluted with beef of negative carcasses.

Further down the food pathway, for raw steak tartare patties, prevalences are almost equal for the three routes of exposure. In contrast to the ground beef batches, the distributions of the weights of the units are equal here. The prevalence at route ‘art 4’ is lowest, presumably because the mean number of animals contaminating a carcass (m_{mean}) is lower: the probability that a carcass gets contaminated by a STEC positive animal is lower in this route.

For these raw tartare patties we differentiate between three age classes: 1-4 years, 5-14 years and 15 years and older. The results for these age classes are given in Tables 5-4 and 5-5. The weights of the tartare patties (W_{tt}) differ between these age classes, with young children eating less tartare on average. Apparently, the effect of these smaller weights on the exposure is quite small.

The distributions of the numbers of cfu per positive steak tartare patty for different exposure routes and age classes are shown in Fig 5-1 and Table 5-6. For all the % STEC O157 contaminated raw steak tartare patties in the Netherlands (that is 0.3% of the total), about 64% is contaminated with a single cfu, and only 7% with more than 10 cfu. The mean number of cfu per positive tartares is largely determined by a small number of heavily contaminated patties and is therefore of limited use as an indicator of mean contamination. The difference between numbers of STEC O157 in ‘industrially’ and ‘traditionally’ produced tartares is (again) explained by the dilution of STEC O157 contamination in large ground beef batches.

Table 5-1 Difference between routes of exposure. The parameters with different values for different routes of exposure are given. All values are expert estimates. (with m_{mean} the mean number of animals from which the faeces contaminates a single carcass, n_{mean} the mean number of carcasses used for a ground beef batch and W_{gb} the weight of a ground beef batch)

	art 10, ind.	art 10, trad.	art 4.
m_{mean}	2.98	2.98	1.61
n_{mean}	50.33	1.44	1.44
most likely W_{gb} (kg)	7.7	370	370
relative production	77.1%	19.4%	3.5%

Table 5-2 Baseline risk model results at the stage of the ground beef batch, for different routes of exposure and the means for the Netherlands (NL).

	art 10, ind.	art 10, trad.	art 4.	NL
prevalence	26.2%	1.7%	1.1%	20%
mean cfu/pos batch	104	398	591	178
mean batch weight (kg)	467	8	8	362

Table 5-3 Baseline risk model results at the stage of raw steak tartare patties, for different routes of exposure and the means for the Netherlands (NL). (Pos. tartare = STEC O157 contaminated steak tartare patty)

	art 10, ind.	art 10, trad.	art 4.	NL
prevalence	0.29%	0.30%	0.21%	0.29%
mean cfu/pos. tartare	3.4	670	1700	190
pos. tartare with one cfu STEC O157	72%	38%	36%	64%

Table 5-4 Prevalences of positive steak tartare patties per age class and route of exposure and the means for the Netherlands (NL).

	1-4	5-14	15+	NL:
art 10 ind	0.19%	0.28%	0.29%	0.29%
art 10 trad	0.24%	0.29%	0.30%	0.30%
art 4	0.17%	0.20%	0.22%	0.21%
NL	0.20%	0.28%	0.29%	0.29%

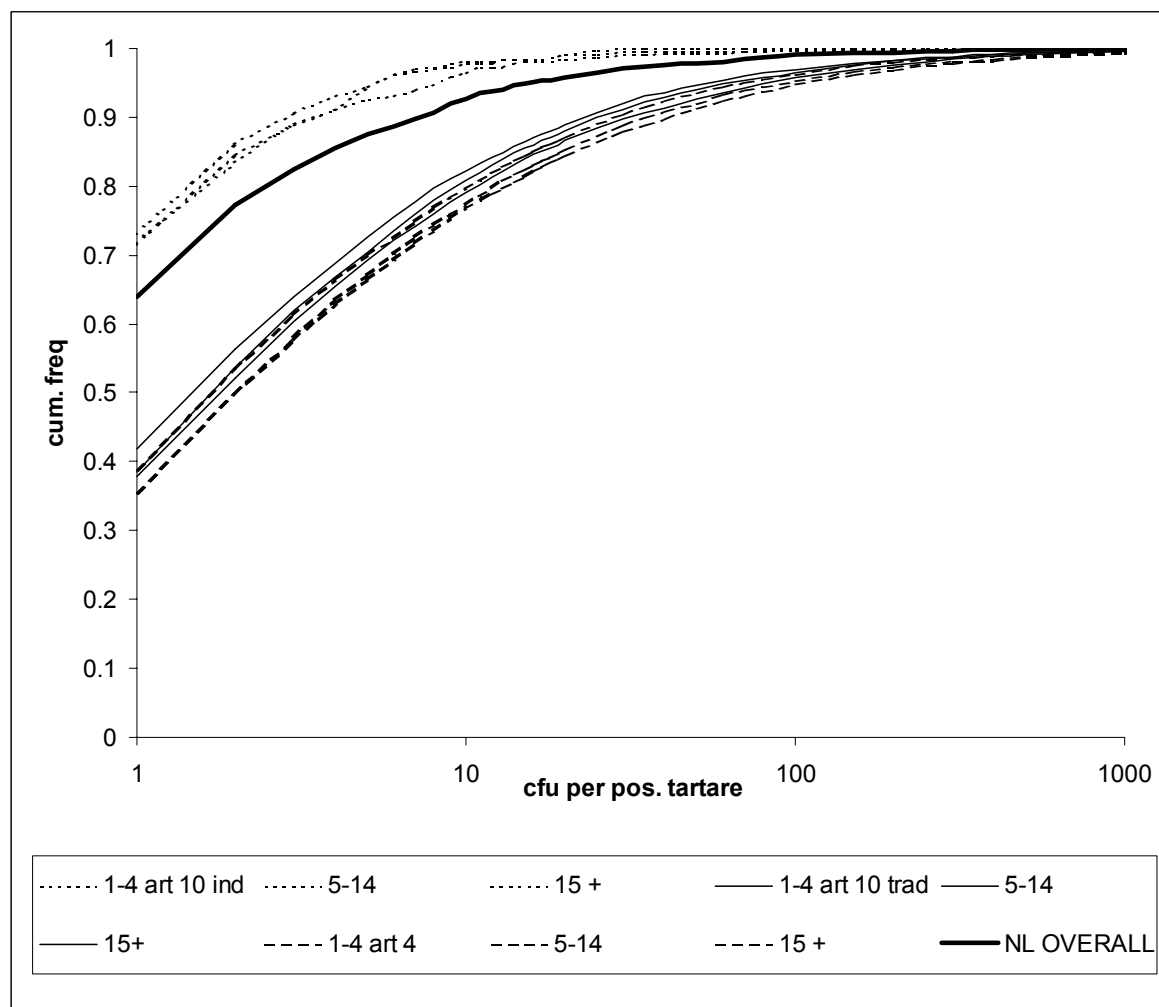


Figure 5-1 The distribution of the levels of STEC O157 in contaminated raw tartare patties. The cumulative frequencies of the number of STEC O157 cfu per tartare patty are given for each age class and each route of exposure. The fat line represents the result for all steak tartare patties in the Netherlands. Note the logarithmic horizontal axis. Most tartare patties contain one or only a few cfu.

Table 5-5 Mean numbers of cfu per positive raw steak tartare patty per age class and route of exposure and the means for the Netherlands (NL).

	1-4	5-14	15+	NL:
art 10 ind	2.6	3.1	3.5	3.4
art 10 trad	550	500	700	670
art 4	1100	1400	1800	1700
NL	150	150	200	190

Table 5-6 The distribution of the numbers of STEC O157 cfu in contaminated raw steak tartare patties. For the three routes of exposure and all tartares in the Netherlands the relative frequencies of five classes of the contamination level are given. Numbers represent the variability as predicted by the baseline model. See Fig 5-1 for illustration.

cfu per tartare patty	art. 10 industrial	art. 10 traditional	art. 4	NL
1	71.6%	38.2%	35.6%	63.9%
2-10	25.1%	41.3%	41.4%	28.8%
11-100	3.2%	16.4%	18.0%	6.3%
101-1000	0.0%	3.7%	4.5%	0.9%
> 1000	0.0%	0.5%	0.6%	0.1%

After incorporation of the preparation model (food pathway step 8), and the probability of consumption per age class and preparation style, the exposure model is linked to the dose response model. This yields a prediction of the number of persons in the Netherlands that is infected with STEC O157 per year. Results are given in Table 5-7. It shows that the attack rate (the rate of infection per person per year in each age class, calculated by dividing the expected number of infected people in each age class by the number of inhabitants, I_{ac}), is largest for the youngest age class. This may be explained by a relatively large probability of consumption of steak tartare combined with a larger probability of infection by a single cell (r) in the younger age classes.

As found in the Japanese outbreak (Shinagawa 1997), it is assumed that 55% of the infected peoples get ill. Then, with the baseline risk model, it is predicted that per year 1284 people in The Netherlands get ill due to STEC O157 ingested by consumption of steak tartare patties, that is an incidence rate of 8 per 100,000 person years.

Table 5-7 Results of the risk characterisation. The predicted attack rate, number of infections per year in the Netherlands and cases per year in the Netherlands for the whole population of 1 year and older.

	1-4	5-14	15+	NL
attack rate (infections per person per year)	0.020%	0.016%	0.015%	0.015%
infected (per year)	157	301	1877	2335
cases (per year)	86	168	1030	1284

5.2 Evaluation of alternative scenario's

In a scenario analysis the baseline risk model with default values of the parameters can be compared with alternatives. In general, such a scenario analysis can serve different goals. In a sensitivity analysis, the sensitivity of the model output for small deviations in parameter

values can be compared between parameters. This is particularly helpful as a tool to find out for which parameters precise estimation is (not) important, or at which parameters intervention should be aimed (see e.g. Nauta et al. 2000). In an uncertainty analysis, the effect of the (quantified) uncertainty in a parameter on the uncertainty in the model output is evaluated. This may be helpful to analyse how much the uncertainty of different parameters used in the input of the model contributes to the total uncertainty of the output. (Actually, this is what the ‘sensitivity analysis’ in @Risk is doing.) Alternatively it may be used to analyse how much the uncertainty in a specific parameter may affect the assessed model outcome. By thus comparing different parameters, the effect of uncertainty in a parameter on the uncertainty of end result can be studied. The latter method is used here.

For different parameters at different steps along the food pathway, the default value is adapted and the risk model is run. This alternative scenario gives an alternative prediction of the number of ‘STEC O157 by steak tartare consumption’ cases, and a prediction of the prevalence of positive raw steak tartare patties. The choice for the alternative scenario per parameter depended on the parameter characteristics and the available information. For parameters for which the 95% (or 5%) confidence level value was available, e.g. from expert opinion (Table 3-17), these values were taken so the effect of the uncertainty in these parameters could be compared. For those parameters for which no uncertainty interval was available, an other alternative value was selected. This allowed the evaluation of the effect of a disputable model assumption (like the distribution of G_{prc} or the dose-response model chosen), the effect of taking extreme values (like ‘no clustering’ or $m_{mean}=n_{mean}=1$) and a comparison of the different routes of exposure (Fig 3-7).

For each alternative scenario the first part of the risk model was run with 50000 iterations for each of the three food pathways, and the second part for 25000 iterations, as the baseline model.

An overview of the results of this scenario analysis is given in Table 5-8 and Figures 5-2 and 5-3. It shows that the largest increase in incidence is an eightfold increase which results from a tenfold increase of the faecal contamination (in g per carcass), and that the largest decrease results from using a different dose response model (Powell et al. 2000). When the three routes of exposure are compared, it shows that if all steak tartare patties would be the product of the art. 10 industrial route, the incidence is expected to decrease by about 50%. If all tartare patties would be produced by one of the other, more traditional, routes, the incidence would increase two- to threefold. Next, it is remarkable that a slightly different distribution of the variability in growth/inactivation on the carcass (characterised by G_{prc}) has a relatively large effect and that the effects of cell clustering and consumer and retail storage are small.

5.3 Validation

To validate the risk model, the model prediction should be compared with independent data. Unfortunately, data that can be used for that purpose are scarce. It was possible to make a comparison at two points: the prevalence of STEC O157 in raw steak tartares patties as found

in a small microbiological surveillance (De Boer et al. 1997 and Heuvelink 2000a), and the incidence of all STEC O157 infections and clinical cases in the Netherlands as derived from epidemiological data (Havelaar et al. 2001).

The only source of data on prevalence of positive steak tartare patties is the result of De Boer et al. (1997) and Heuvelink (2000a) who found one positive in a surveillance study on 82 steak tartare patties. Using the Beta distribution with a uniform prior to estimate the uncertainty interval around the $1/82 = 1.22\%$ point estimate, Beta(2, 82), yields a 95% confidence interval for the prevalence of STEC O157 contaminated raw tartare patties between 0.29% and 6.53 %. This is rather broad, with the baseline risk model prediction 0.29% lying just on the 2.5% level.

However, when comparing the 'surveillance prevalence' and the 'risk model prevalence', note that the risk model predicts a large fraction of patties with only one cfu, where the detection limit in the microbiological analysis is probably much higher than 1 cfu. This implies that one would expect that the prevalence predicted by the model would be higher than the prevalence found in a surveillance study. In contrast, we find it is lower. With a detection limit of for example 10 cfu, the fraction of positive raw tartare patties in a surveillance as predicted by the baseline risk model would be much lower, about 0.02%. The probability of finding one or more positive tartare patties in a sample of 82 would then be 1.6%.

Apparently the risk model underestimates the risk when prevalence of STEC O157 contaminated tartares is considered.

Havelaar et al. (2001) have estimated the incidence of STEC O157 associated gastro-enteritis, based on combined data from several epidemiological studies: population based surveillance, sentinel studies in general practices and laboratory surveillance. The results of this study are summarised and compared with the outcomes of the risk model in Table 5-9 and Figure 5-4. The epidemiological studies indicate that, on average per year, approximately 2400 infections with STEC O157 occur in the Dutch population of 1 year and older, resulting in approximately 2000 cases of gastro-enteritis. There is a wide margin of uncertainty in these estimates: 90% confidence interval 85-6500 cases per year, median 1200. The risk model predicts an average incidence of infections of approximately 2300 of which 1300 lead to gastro-enteritis. Thus, more than 95% of all infections and 65% of all illnesses by STEC O157 would be caused by steak tartare consumption. Note that the relative contribution of steak tartare differs for the three age classes, and that the risk model predicts more infections in the 15+ age class than are actually observed in the population. We have no information to support the finding that the majority of STEC O157 cases in the Netherlands are the consequence of steak tartare consumption. Actually, other sources such as direct contact with animals and secondary transmission are known to be significant risk factors for STEC O157 infection, and other food sources than steak tartare, such as hamburgers or ox-sausages are probably important as well. Therefore, in contrast to the prevalence of STEC O157 in raw steak tartare patties, the risk model now seems to overestimate the risk.

Table 5-8 Results of the scenario analysis. A list of alternative scenario's for which the risk model was recalculated, sorted by the predicted number of cases. In each alternative scenario one or a few parameter values are adapted. The resulting prediction for the prevalence in raw steak tartare patties and the incidence (the yearly number of cases in the Netherlands resulting from steak tartare consumption) are given. *The total incidence in the Netherlands (that is of *all* STEC O157 cases) is predicted on the basis of independent epidemiological data (Havelaar et al. 2001 see 5.3). The prevalence of positive tartares is the mean estimate from surveillance data (De Boer et al. 1997 and Heuvelink 2000a, see 5.3)

alternative	parameter change	cases per year	P_{tt} (before prep)
Powell DR	$r \sim \text{Beta}(0.221, 8800)$	17	0.29%
art. 10 industrial	exposure route ind. art 10: 100%	560	0.29%
Extreme clustering	$b_2 = b_4 = b_6 = 0.11$	800	0.12%
equal rel. contributions	$b_1 = b_5 = 100$	1000	0.26%
f_{wd} well done at 95%	$f_{wd} = 41.2\%$	1010	0.29%
P(cons) classic	$P_{cons, ac, pst}$ as 'classic' in Table 3-20	1230	0.29%
no correl. n - W_{gbb}	$r_{corr} = 0$	1240	0.30%
DEFAULT	-	1280	0.29%
No clustering	$b_2 = b_4 = b_6 = \infty$	1330	0.45%
n 5%	$n_{mean, ind} = 30.07,$ $n_{mean, trad} = 1.01$	1420	0.30%
one animal: $m = n = 1$	$m_{mean} = 1, n_{mean} = 1$	1430	0.10%
storage	$T_{mean} = 10, t_{mean} = 48$	1490	0.30%
$G_{prc} = 0$	$G_{prc} = 0$	1490	0.33%
$W_{tt} = 100$	all $W_{tt} = 100$	1520	0.31%
f_{wd} well done at 5%	$f_{wd} = 2.56\%$	1540	0.29%
a Cassin	$\log(a) \sim \text{Normal}(-0.5, 0.9)$	1650	0.31%
m 95%	$m_{mean, art 10} = 5.73,$ $m_{mean, art 4} = 1.44$	1800	0.52%
VALIDATION	-*	1968	1.22%
$P_f = 0.02$	$P_f = 0.02$	2260	0.56%
Hypgeom DR	$r_{1-14} \sim \text{Beta}(0.102, 2.337)$ $r_{15+} \sim \text{Beta}(0.067, 3.017)$	2460	0.29%
low art 10 ind.	art 10 ind. : 38.5% art 10 trad. : 58.1% art 4: 3.5%	2540	0.29%
inactivation at 95%	$f_{surviv, med} = 73.8\%.$ $f_{surviv, wd} = 6.24\%.$	2840	0.29%
art. 4	exposure route art 4: 100%	3290	0.21%
art. 10 traditional	exposure route trad. art 10: 100%	3830	0.30%
$G_{prc} = \text{Cassin}$	$G_{prc} \sim \text{Triang}(-2, 0, 5)$	4250	0.60%
$a_{max} = 100$	$a_{max} = 100$	10180	1.09%

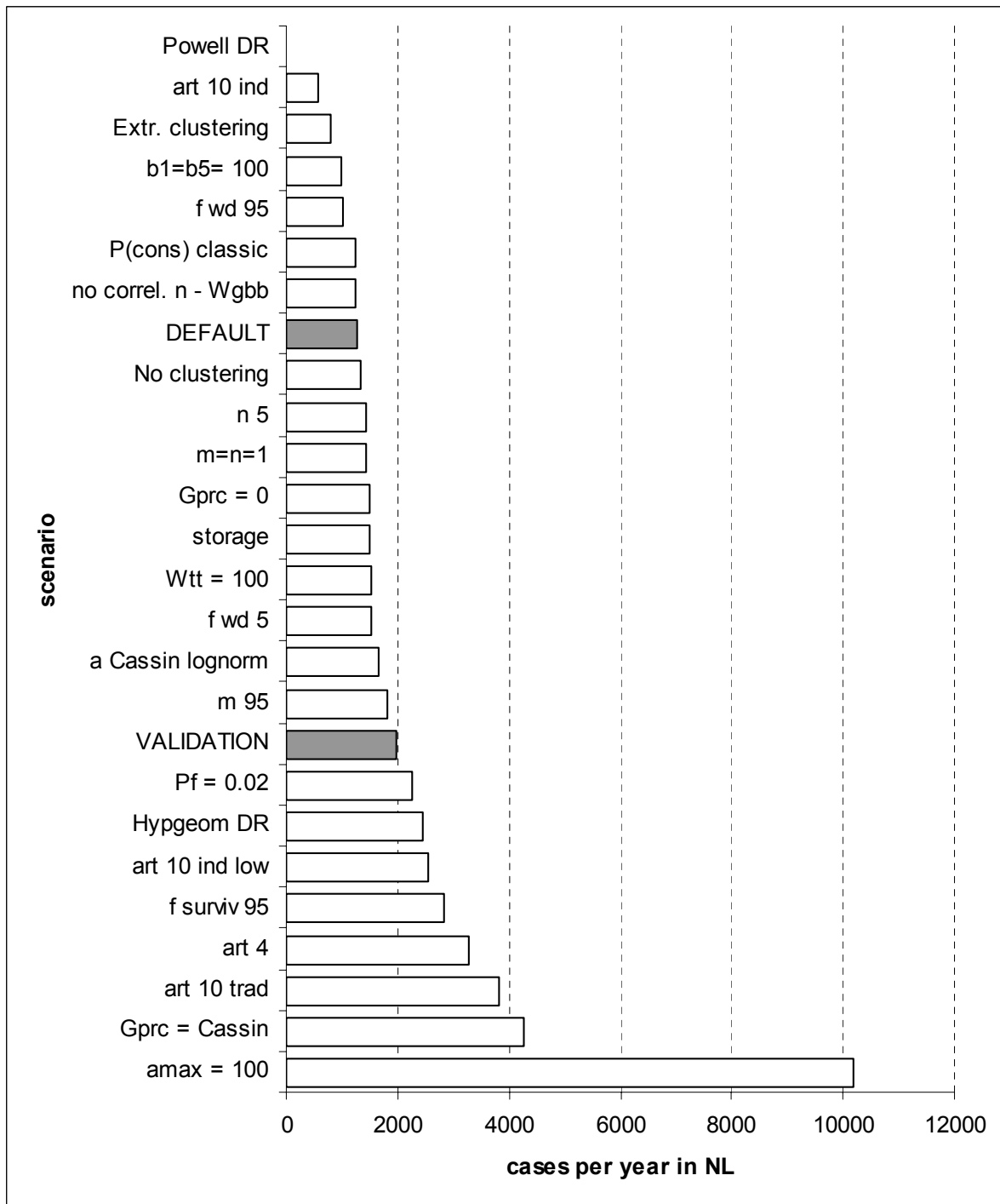


Figure 5-2 Comparison of the predicted incidences (STEC O157 cases per year in the Netherlands) for different alternative scenario's, compared with the results of the baseline model and the predicted total incidence in the Netherlands, based on epidemiological data (see 5.3).

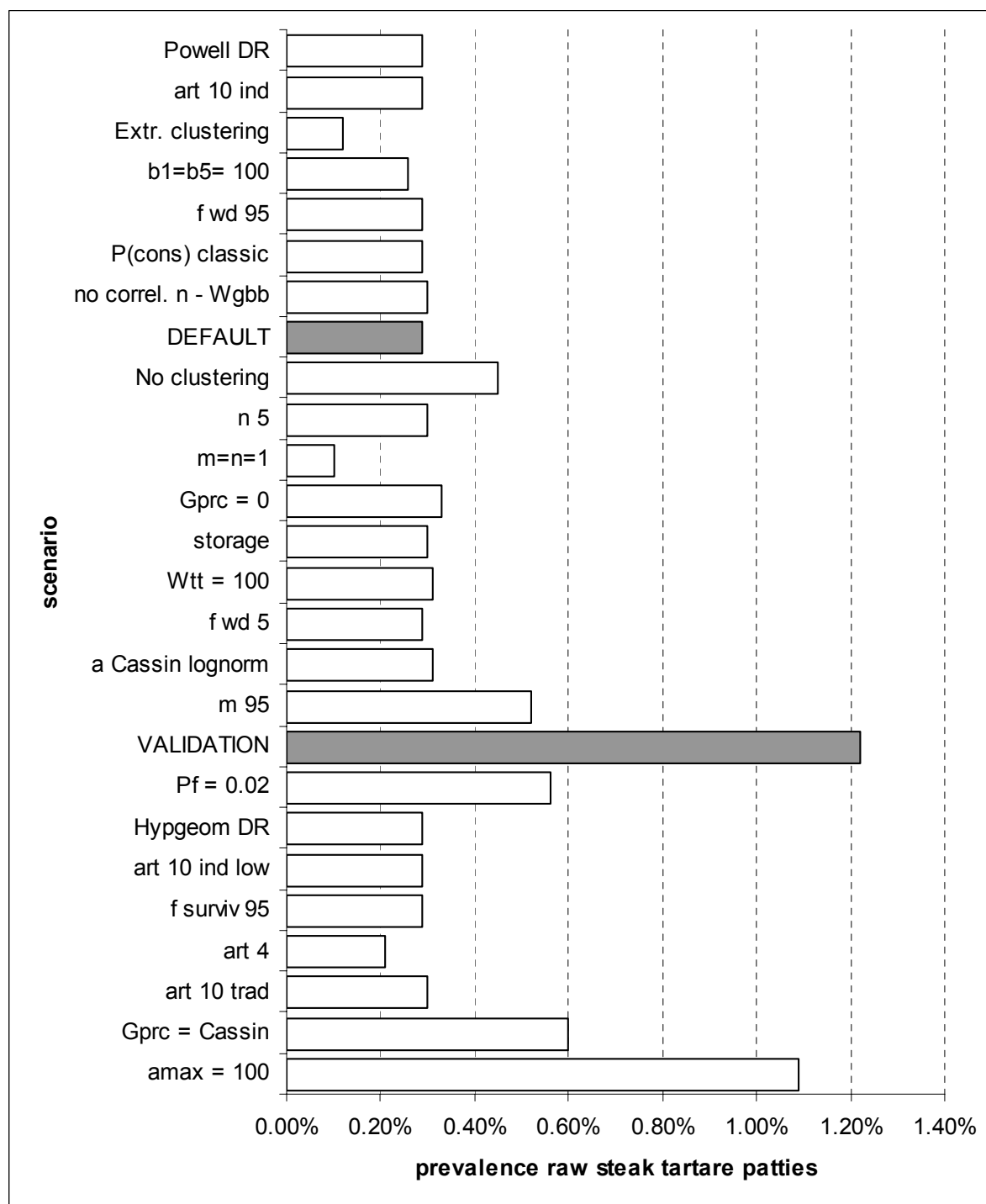


Figure 5-3 Comparison of the predicted prevalence of contaminated raw steak tartare patties for different alternative scenario's, compared with the results of the baseline model and mean estimate based on surveillance data (De Boer et al. 1997 and Heuvelink 2000a, see 5.3). For comparison, the scenario's are sorted as in fig 5-2.

Table 5-9 Overview of the predicted number of STEC O157 infections and the number of cases in the Netherlands, per age class and for the total Dutch population. The mean estimates of Havelaar et al. (2001), that are based on epidemiological data, are compared with the results of the baseline risk model. Havelaar et al. (2001) use the age category 0-4 as the youngest age class, and, due to the absence of consumption data, this study uses 1-4 as the youngest age class. Therefore the Havelaar et al. (2001) results are adapted by taking 80% of the 0-4 age class number. (Numbers given in italics).

	<i>0-4</i>	<i>1-4</i>	<i>5-14</i>	<i>15+</i>	<i>NL</i>	<i>NL</i>	<i>1+</i>
Infections							
Havelaar et al. (2001)	630	<i>504</i>	428	1469	2527	<i>2401</i>	
risk model		157	301	1877		2335	
Cases							
Havelaar et al. (2001)	516	<i>413</i>	351	1204	2071	<i>1968</i>	
risk model		86	168	1030		1284	

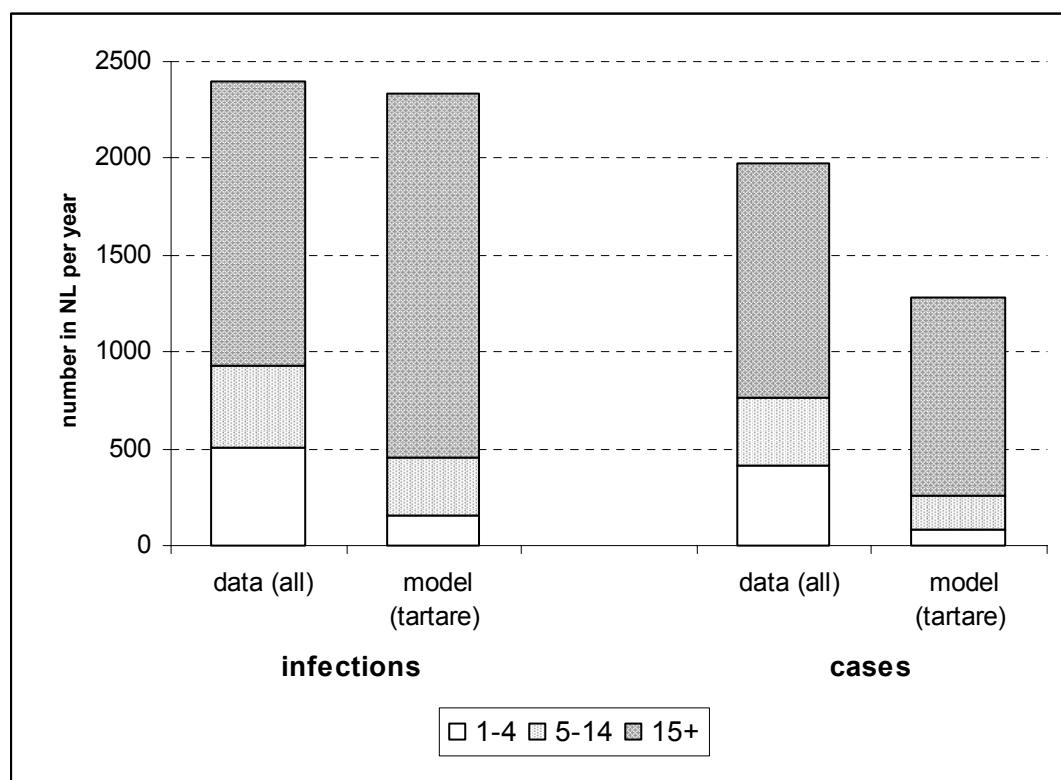


Figure 5-4 A comparison of the results of Havelaar et al. (2001) that are derived from epidemiological data (data), and the risk model predictions (model) for different age classes. Note that the 'data' results are for all STEC O157 infections and cases in the Netherlands, and the 'model' results for steak tartare only. Apparently the risk model overestimates the number of infections and cases.

6. Information campaign

6.1 Introduction

One of the measures proposed in sections 2.5 and 2.7 to reduce the public health risk of STEC O157 was to use an information campaign to influence consumer behaviour with respect to tartare. As stated in section 2.7, time limitations were the main cause of not including calculations on the effect of information campaigns in this project. However, as will become clear from the information in this chapter, this might have been a difficult task even if time would not have been limited. It appears that the effect of an information campaign depends on many factors and quantitative data on effects are very scarce. Although effects of information campaigns were not included in this risk assessment, we still include the obtained information in this report, as an impression of available state of the art information. Also, this information is a useful basis for further research within the framework of future risk assessment projects.

The first approach followed by the authors was to describe the consumer phase with a simple model, with the idea that influencing the behaviour could be quantified by effects on specific parameters. This model is depicted in Figure 6-1. Two routes are distinguished:

- Buy tartare at the butcher - transport home - store in refrigerator - preparation - consumption;
- Buy tartare (roll) at the snack bar - consumption.

Three research groups (Communication and Innovation studies, Wageningen; Nutrition Centre, Den Haag; RIVM/Department for Public Health Forecasting) were visited to discuss a number of aspects, using a/o figure 6-1. Major aspects to be discussed were:

- What is the state of the art knowledge of consumer behaviour;
- Is it possible to make a quantitative estimation of the effect of a measure;
- How can the effect of a measure be expressed (e.g. percentage of people changing their behaviour, the extent to which people change their behaviour, etc.);
- Which measure is more effective: an information campaign for the general public or education at the primary or secondary school;
- What is more effective: to aim an information campaign at one or at more aspects; to aim an information campaign at consumption reduction or at changes in other behaviour aspects;
- What is the aspect for which change will have the largest effect.

Information on these aspects was obtained to a varying extent. The information obtained is given below, subdivided into the above mentioned three research groups.

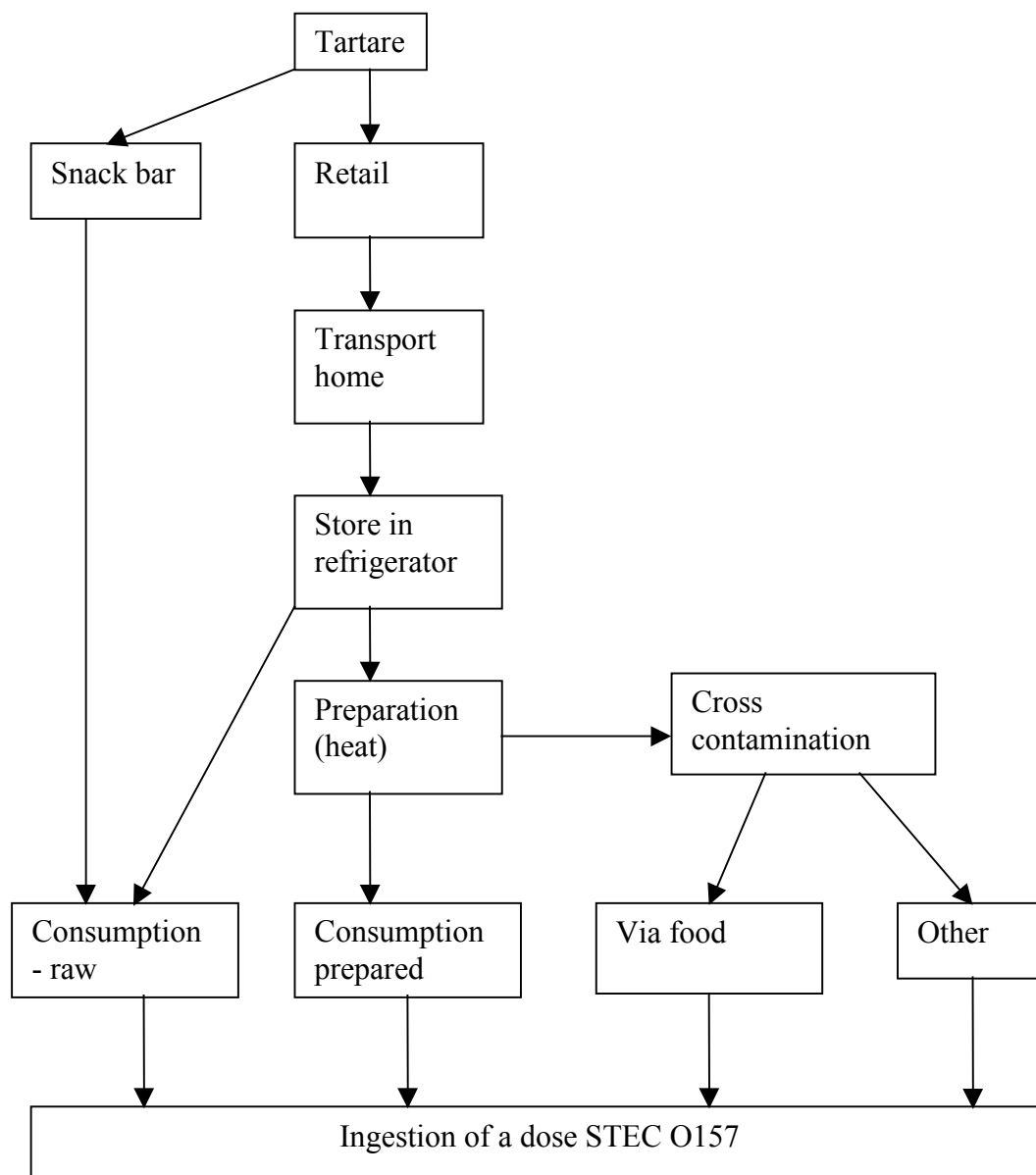


Figure 6-1 Simple model for the consumer phase

6.2 Communication and Innovation Studies Wageningen

One of the main objectives of this group (Wageningen University / Social Studies / Communication and Innovation studies) is communication to the general public. Using a simple model for the events in which consumer behaviour plays a role shows that there is a distinction between two things: influencing peoples behaviour and to what extent do people change their behaviour.

The success of an information campaign depends mainly on two aspects:

- 1) There must be an alternative. People find risks more of interest if they can do something about it. Fundamentally, people are receptive.
- 2) The proposed measures must be concrete.

An important condition is that the issue troubles people, outbreaks being an example. This explains why *Salmonella* is an issue and the more frequently occurring *Campylobacter* isn't. The specific case of STEC O157 in tartare is favourable, because there are alternatives and these are concrete (e.g. bring the bought product rapidly to the refrigerator). It is however difficult to predict the effect of an information campaign. In summary: favourable, but difficult to predict.

The effect of an information campaign is difficult to calculate as it depends e.g. on the campaign itself and on the publicity around it (e.g. when there is an outbreak during the campaign). For commercial advertising it is an accepted fact that a campaign has a high risk (i.e. the probability of no or little effect of a campaign is high), although the campaign is in time followed by tracking techniques to judge its effect. The advertisement world rates information campaigns from the government rather low. From the viewpoint of the advertisement world, campaigns from the Health Council of the Netherlands e.g. suffer from a set-up which is analogous to the making of a diagnosis by a general practitioner.

Another problem is that an information campaign has only effect on the willingness to change behaviour, which is the endpoint looked at by this group. The willingness to change behaviour is however badly correlated with the actual behaviour, which is what we really want to influence. The actual behaviour is influenced by things like habits, the environment, concreteness of the alternative.

An information campaign is only a part of the strategy to be followed to achieve an effect on consumer behaviour. Another, really important, aspect is a telephone number people can call with questions. Also important for a good strategy is co-operation with other relevant organisations, such as consumers' organisations and medical organisations. Experience shows that the effect of education at primary or secondary schools is small and thus inferior to an information campaign.

A quantitative approach to the effect of an information campaign is not recommended. A relative approach using an ordinal scale might be better.

6.3 The Netherlands Nutrition Centre

The information below is based on discussions and on the references mentioned.

Information campaign - general

An information campaign will not be successful when it is aimed at the general population. It might be successful when aimed at groups at risk: pregnant women, children. It should be aimed specifically at ground meat. Certain groups are difficult to reach, such as foreigners and groups with low socio-economic status. A campaign should be aimed at information supply and awakening. Television is not a suitable medium to realise a change in behaviour. It cannot in general be decided that it is better to aim an information campaign at one or at more aspects, or whether it is better to aim the campaign at reducing consumption or a different change in behaviour; this depends on the specific objective.

An information campaign must be prepared well. Amongst others, one must define a target group and one should investigate how people think about the subject. Filling in a campaign must be preceded by proper research on your target group. For tartare, knowledge probably is the most important determinant. A much used model for arranging a campaign is the PRECEDE/PROCEED approach (Damoiseaux and Kok 1993). This is a good general book on health education and behavioural change.

Three relevant campaigns can be distinguished:

- a) 'Let op vet'. Period 1991-1995. See e.g. Brug et al. (1993);
- b) 'Goede voeding, wat let je'. Period 1996-2001. See e.g. Voedingscentrum (1999a) and Hazebroek (1997);
- c) 'Eet en Woon – Veilig en Schoon'. Period 1999-2001. See e.g. Voedingscentrum (2000). Part of the project on Hygiene in private households, see e.g. Voedingscentrum (1999b).

Theoretical models for effect evaluation

In this section, three models for effect evaluation are described (based on Hazebroek 1997 and Hazebroek 1999). Variables that are measured in effect evaluation are based on these models. To be able to measure the effect of an information campaign, a zero measurement is necessary.

ASE-model

Behaviour is determined by factors, called determinants. The ASE model (Figure 6-2) uses the determinants Attitude, Social influence and Own effectiveness. Own effectiveness is the extent to which a person thinks that he is capable to show the desired behaviour. The determinants influence directly the intention a person has to show the desired behaviour. Whether the person will show the behaviour can be hindered by barriers and lack of skills. Misconceptions of ones own behaviour is an example of a barrier.

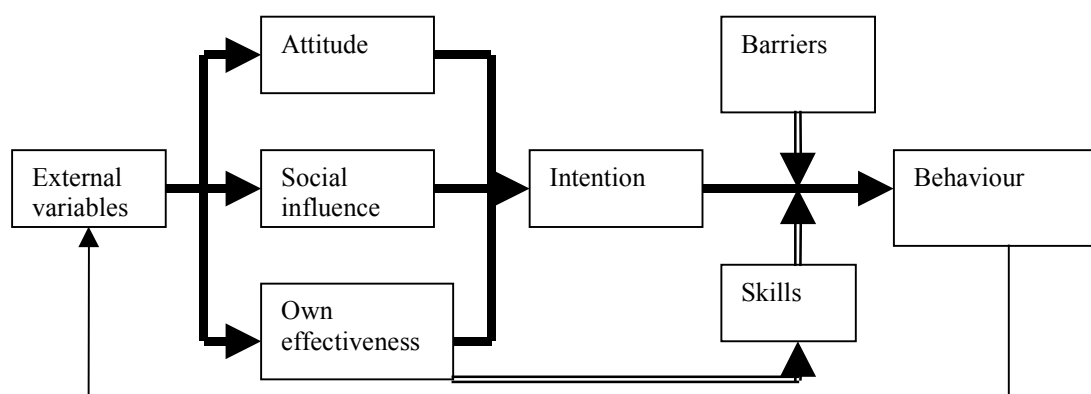


Figure 6-2 The ASE model for explanation of behaviour.

The phase-model of change in behaviour

The phase-model described here is often used to evaluate the effects of nutrition education. It is one of the theories that describe change in behaviour, aimed at explaining change processes in an individual. Figure 6-3 depicts the model. For an information message to lead to a change in attitude, the message must get attention and be understood. A change in attitude can only be achieved if the receiver changes his opinion on advantages and disadvantages of the behaviour as a result of the message. Change in attitude can lead to change in intention, if not hampered by social standards. In order to change behaviour, people must have the possibility to convert from intention to behaviour. The retention of behaviour depends on experiences with the behaviour. The occurrence of expected (positive) effects is important for this.

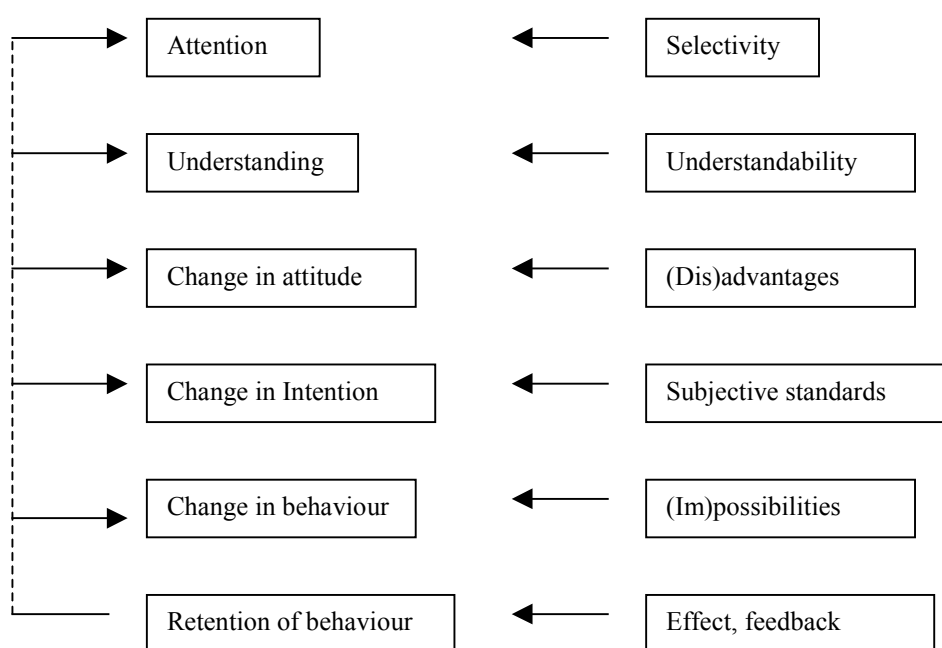


Figure 6-3. A phase model for change in behaviour by education.

The stages-of-change model

The stages-of-change model describes change in behaviour as a dynamical, non-linear process. In this model the process of change in behaviour has been arranged in five stages:

1. Precontemplation: the person has never considered a change in behaviour;
2. Contemplation: the person considers a change in behaviour;
3. Preparation: the person prepares a change in behaviour;
4. Action: the person is actively changing behaviour;
5. Retention of behaviour: the person tries to retain the new behaviour and prevent relapse.

Persons can get a relapse at any moment in the cycle and end up in an earlier phase, after which the following phases are passed through again.

Zero measurement

As an example of a zero measurement, we give here the phrasings of questions used in the campaign 'Goede Voeding, wat let je' (Hazebroek 1997).

1. What are the determinants of behaviour for consumption of fat, bread, etc.;
2. What is the mean level of consumption of energy, fat, etc.;
3. How high is the frequency of misconceptions of consumption of fat, vegetables, etc.;
4. In what phase of behavioural change for consumption of less fat and vegetables is the target group;
5. Are there differences between subgroups.

Effects - general

It is difficult to measure the effect of a campaign in terms of change of behaviour. First question to be answered is what to measure. But besides this, there is a difference between what people say they do and what they actually do. In any case it is important to do a 'zero' measurement. The change in behaviour can be realised by a campaign, but using intermediates is also an (additional) option. The effect of a campaign will be a long term effect.

As regards to the effectiveness of education at school compared to an information campaign, it is thought that education at school will not be a success. Arguments for this belief are that the school program is already very full and that pupils have little interest in the subject 'care'. It should be realised that feeding behaviour is especially difficult to change, compared to other types of behaviour. It is even more difficult than smoking, because it is not necessary to smoke, but it is necessary to eat. In a feeding campaign, it is difficult to name short term advantages.

The 'Goede voeding, wat let je' campaign

The objective of the campaign 'Goede voeding, wat let je' (translation 'Good nutrition, why not?') was set too high initially. For this campaign, originally percentages were set which were to be reached in time for the different phases of the phase-model of change in behaviour (Hazebroek 1997). It is now realised that this is not realistic and that it is difficult to change behaviour. It is very much a long term process which is difficult to measure. One of the many problems is that there are several organisations with the same message and it is not possible to assign the result to one organisation. One has turned now to determinants of behaviour. The 'Goede voeding, wat let je'-campaign is now aimed specifically at its two most important determinants:

- Own effectiveness (also called skills);
- Misconceptions (an important barrier).

An example of a misconception are people who think they eat enough vegetables and fruit, whereas they don't. Using the two determinants above indicates the direction of the campaign.

Effect measurement of the 'Goede voeding, wat let je'-campaign is or will be done by the following means:

- Execute an activity in Zoos and questioning at the exit; questioning in McDonalds; and by telephone questions on supermarket visits.
- For this campaign, a zero measurement (Hazebroek 1997) and an intermediate measurement (Hazebroek 1999) were done. A final measurement will be done also.
- Additional evaluation research on the reach of the campaign is done on a yearly basis. Campaign familiarity and reach can be used as an upper limit of effect and, if this determinant itself is the aim, for quantification.

The 'Eet en Woon - Veilig en Schoon' campaign

The 'Eet en Woon - Veilig en Schoon'-campaign (translation 'Eat and Live - Safe and Clean') is part of the project Hygiene in Private Households. In this project, a hygiene code for private households was formulated (Voedingscentrum 1999b) and a zero measurement on knowledge, attitude and behaviour with respect to hygiene in and around the house was performed (Voedingscentrum 1999c). The hygiene code was meant for intermediates, such as dieticians, consumer bureau's and the business community. It was however bought by many consumers. For the 'Eet en Woon - Veilig en Schoon'-campaign, an effect measurement is planned in 2001, with a sample size of 300 people. As changes in behaviour are difficult to measure or not expected, such measurements will be aimed at changes in intention or attitude. The present campaign is primarily aimed at the kitchen and at children and pregnant women. A folder and a writing pad were developed and spread by Felicitas congratulation service, promotion teams, the Femina fair, butchers. At present there is broadcasting time in Lunch TV at TV2. There will be an extension to other hygiene themes. Moisture will be an important theme. Publications will be placed in daily papers, women's magazines, etc. Maybe an information game for the computer will be made. The connection between the nine objectives of the Hygiene code must remain clear. Key words are time, temperature and moisture.

The 'Let op Vet' campaign

The effect of the 'Let op Vet' campaign (translated freely 'Take Care with Fat') was investigated (Brug et al. 1993, Hemel et al. 1994a and Hemel et al. 1994b). This campaign was executed from 1991-1994 and its aim was to reduce fat consumption with 10 %. For the campaign, mass medial campaign materials were used and there was co-operation with intermediates, especially the food retail trade.

- The 1991 campaign was widely known (60 %). It resulted in an increase of interest in the subject (from 79 to 84 %). There was an increase in the percentage of people stating that fat consumption in the Netherlands is too high. There was no change in the awareness of ones own fat consumption;
- The 1992 campaign was known by 40 % of the people. No positive campaign effects were found. A negative shift was observed in many aspects compared to the post 1991 campaign measurement;
- The 1993 campaign was known to 32 % of the people. Again no positive effects were found;

- The sale of less fat products seems to increase in 1991/1992 compared to 1990, but the effect is not entirely clear;
- The 1994 campaign was known by 47 % of the people, which is an increase of 13 % compared to prior to the campaign. There is no significant change in the behaviour or determinants of behaviour (attitude, own effectiveness, social influence, intention) of the target group. A quantitative analysis of the ASE-model is given.

Effects - quantitative

It is clear from the information given above that quantitative data on the effect of information campaigns are very scarce. Miscellaneous written material obtained from the Netherlands Nutrition Centre contained quantitative data that give at least an impression of the size of effects that are to be expected from an information campaign. These data are given below. Using these data for estimating effects must of course be done with great care.

For the average effect of a mailing, the following numbers can be given, setting the target group at 100 %:

Heard of: 70 %
 Looked at: 50 %
 Purchased: 30 %
 Used: 15 %
 As intended: 5 %

The effect of a pre-test Fit & Fun: 7.1 % of non-active young persons is thinking seriously about doing more on sports as a result of Fit & Fun.

Target percentages, which are a realistic estimate of the reach of a campaign, can also be used for estimation of campaign effects. Such percentages, taken from a subsidy request, are given below. They are subdivided into the categories education, mass media and facilities.

I. Education

1. Supermarket

Consumption tests	Seen by:	50 %
	Filled in by:	20 %
Stickering cool fresh vegetables	Seen by:	30 %
Jingle	Heard by:	50 %
	Able to reproduce it:	20 %

2. McDonalds

Moodboard:	Seen by:	50 %
Snack test on placemat	Seen by:	50 %
	Done by:	20 %

3. Zoos

Preparation demonstration	Seen by:	30 %
	Increased confidence	
	in skill 1:	40 %
	Increased confidence	
	in skill 2:	30 %

Information panel	Seen the message:	20 %
Feeding of the animals	Heard the message	15 %
II. Mass media		
1. Television	Seen by:	35 %
2. Information supply to intermediates	Informed:	50 %
III. Facilities		
1. McDonalds	Target group has knowledge 1:	50 %
	Target group used this knowledge:	15 %
2. Supermarkets	Percentage of product 1 stickered:	20 %
3. Zoos/amusement parks	Sign manifesto:	55 %

Tartare

Cross contamination will play a minor role for tartare. It will mainly take place via the fork. The fact that tartare is not cut into pieces reduces cross contamination. Useful quantitative data in this respect could include the number of micro-organisms on hands before and after washing.

One measure to reduce public health risk associated with tartare is a change in the way tartare is prepared. This will however not be accepted by the general public: tartare is preferred partially raw on the inside. Maybe groups at risk are an exception to this. An alternative measure would be to discourage the consumption of tartare.

If the whole route is considered (Figure 6-1), at present storage in the refrigerator (time, temperature) seems the most important aspect. However, it is clear that more knowledge is necessary to determine the relative importance of aspects.

6.4 RIVM/Department for Public Health Forecasting

Effect of interventions

Since the effect of only an information campaign is doubtful, it could be considered to include some other interventions additional to the information campaign. Interventions on a specific targetgroup in a frequently visited setting (for example a supermarket or a school) and with a more personal approach could give an important contribution additional to a information campaign. For example the anti-smoking programs for young people at school contain such frequent and personal offered intervention-activities. Half of the anti-smoking programs for young people show a significant reduction in the percentage that starts smoking. For these successful programs, it was found that in the experimental groups (after an anti-smoking program) the mean percentage of smokers amounts 15.6%, while on average 22.3% of the control group (without anti-smoking program) is smoking (Aarts et al. 1997).

The 'Let op Vet'-campaign of the Nutrition Centre was evaluated by TNO voeding (Netherlands Organisation for Applied Scientific Research - Nutrition and Food Research) and little change in attitude was found. In the Dutch Nutrition Surveillance System (VCP) a decrease in fat consumption was visible, but this was due to a change in product composition.

A point of different nature is that for a project in which the effect of measures on microbiological public health risk is investigated, two disciplines are necessary: microbiology and information science.

Measures

Three types of measures (scenarios in VTV terminology) are distinguished:

- a campaign using the mass media and folders;
- a campaign specifically for the youth;
- using environment factors (e.g. increase tax, forbid selling below a certain age, reducing the number of outlets, cooling bags in a supermarket).

Further considerations with respect to measures are:

- Aiming at a target group is most successful;
- Co-operation of a general practitioner also has effect;
- Forbidding tartare in snack bars seems not a realistic option.

7. Discussion

7.1 The public health risk of STEC O157 in the Netherlands

7.1.1 Health risk estimation

The baseline risk model predicts about 1300 cases of STEC O157 associated illness due to steak tartare consumption, per year in the Dutch population for persons from one year of age and older (incidence rate 8 in 100.000). As shown in Table 5-9 and Figure 5-4, this prediction is close to the independent estimate of Havelaar et al. (2001), who estimated a mean total of about 2000 cases per year (incidence rate 13 per 100.000). The latter is the total number of cases, so including cases due to other causes than steak tartare consumption. Would these estimates be correct, then steak tartare would be the main route of transmission of STEC O157, causing about 65% of the cases. In that case public health intervention aiming at this product would be very effective. One needs, however, to carefully consider the uncertainty associated with both estimates before drawing conclusions. In the study of Havelaar et al. (2001) uncertainty is quantified by a 90% confidence interval between 85 and 6500 cases per year. The median of the uncertainty distribution is 1200 cases per year. The uncertainty distribution was highly skewed and the probability that the mean of 2000 cases per year underestimates the true incidence is only 33 %.

The risk model estimate of steak tartare related cases is very uncertain. Sources of uncertainty lie in the food pathway description, in parameter estimates and in the models used. The food pathway description in the risk model is considered representative for the steak tartare consumed in the Netherlands. It is however a simplification of the complex beef production pathways characterised by a variety of cattle types, slaughtering practices, free international trade, storage and transport, retail and consumer practices. In the model structure, important (unknown) details may have been overlooked. Also, many model parameter estimates are based on expert opinion, which may be biased, others are based on potentially unrepresentative data, e.g. from foreign countries. Quantifying the total uncertainty was not attempted in this study because the necessary data were not available. As a consequence, a confidence interval around the risk estimate cannot be given. Scenario analysis, in which the effect of uncertainty in single model parameters is analysed, shows that the uncertainty of the model result is large. In most scenarios, in which the value of only one parameter was adapted, the incidence estimate varied between 1000 and 4000 cases per year.

Hence, considering the uncertainty in the two independent estimates does not change the conclusion that a major part of all STEC O157 cases is caused by consumption of steak tartare. In fact, this conclusion is only strengthened because the uncertainty analysis indicates that the mean of the epidemiological estimate is relatively high whereas the baseline risk estimate is relatively low, compared to many other scenarios. Yet, epidemiological studies

have indicated that factors such as secondary transmission and direct contact with animals play an important role in the transmission of STEC O157. Furthermore, steak tartare is not the only beef product that is consumed raw or partly cooked.

One possible cause for overestimation of the risk estimate might be the dose-response model. We based our model on data from a single outbreak in Japan, because it was the only available source of data involving direct observations in humans. It predicts a probability of infection per single cell of 0.93% and uses the finding that 55% of the infected people get ill to predict a probability of illness of 0.5%. Other STEC O157 risk assessments have used indirect estimates, using dose-response data from related bacteria or from experiments with laboratory animals as proxies (see Table 7-1). A detailed discussion of these models will be published elsewhere (Takumi and Teunis, *in preparation*). Table 7-1 shows that the dose-response model used in our study clearly predicts a higher probability of illness than any of the models used by other authors. This might be related to the fact that the strain involved in the Japanese outbreak was exceptionally virulent, for instance because the bacterial virulence was enhanced by the acidity of the dressing or other factors. Clearly, the dose-response model is a major uncertainty in the risk assessment, which can only be resolved by collecting additional human data. Due to the severity of the illness associated with STEC O157, it is unlikely that such data will ever be obtained from volunteer experiments. Thus, active outbreak investigations are an important source of information for microbiological risk assessment.

Table 7-1 A comparison of Dose-response models for STEC O157. The model derived in this study predicts the highest probability of illness per single cell ingested.

Model	Bacterial species	Host organism	Probability of illness per single cell *	Reference
Exponential	<i>E. coli</i> O157	Man	0.005	This study
Beta-Poisson	<i>Shigella dysenteriae</i> and <i>S. flexneri</i>	Man	0.001	Cassin et al. 1998
Exponential	<i>E. coli</i> O157	Rabbit	0.00000006 ⁰	Haas et al. 2000
Beta-Poisson	<i>S. dysenteriae</i> and enteropathogenic <i>E. coli</i>	Man	0.00003	Powell et al. 2000

*Probabilities given for the Beta-Poisson model are mean values of the underlying Beta distribution.

⁰Probability of infection.

7.1.2 STEC O157 in steak tartare patties

Remarkably, the exposure model predicts that a large number of raw steak tartare patties contaminated with STEC O157 contains one cell (or cfu) only (mean default estimate 63%). One cell in a patty of about 100 g will not easily be detected, so this result will not easily be validated experimentally. On the one hand it is the advantage of modelling that one gets insight in the prevalence of such an extremely low dose. The single hit dose response model described in chapter 4 predicts that one cell is relevant, as it gives a probability of infection of about 1 in 100. On the other hand 'one cell' is not as easily defined as it may seem. The fact that microbiologists use the term 'colony forming unit' (cfu) is not without reason, as a 'cell' is not easily distinguished by many microbiological methods used. Besides, microbiological models on growth and inactivation are usually constructed on the basis of data from large populations of cells. This is for practical reasons: it is difficult to measure small population sizes. It is however uncertain whether those models for large populations are equally valid for (very) small populations. There are strong indications that for example the lag phase duration may on average be longer for (very) small populations, due to stochastic effects (Baranyi 1998, Augustin et al. 2000). Next, stochastic birth and death effects may become important in small sized populations. Life and death of a single cell will depend on its micro-environment, which may be highly variable and not under control in foods. In the risk model we tried to incorporate stochastic effects in growth and inactivation models by strictly calculating in whole cell numbers and applying simple birth and death process models (see section 3.3.3). As a whole, however, the uncertainty in the behaviour of single cells and the high predicted frequency of single cell exposure will add significantly to the total uncertainty of the model predictions.

An interesting finding is that the predicted prevalence of STEC O157 in raw steak tartare patties is too low to explain the 1 in 82 (1.2%) as found by De Boer et al. (1997) and Heuvelink (2000a). The risk model estimates this prevalence at 0.3%, with a majority of patties containing one cfu only. This *lower* estimate is surprising, as in a microbiological test the probability of detection of one cfu in a patty will be small. It is therefore expected that the prevalence predicted by the model is remarkably *higher* than the prevalence of patties positive in microbiological tests. The one positive found by De Boer et al. (1997) and Heuvelink (2000a) may be a 'lucky shot', but statistically it is unexpected if the risk model estimate is correct.

Next, this *underestimation* of the risk model is contradicted by the potential *overestimation* of the number of cases due to steak tartare consumption as discussed above. This may imply that the risk model uses unrealistic assumptions in the trajectory of the food pathway between raw tartare at retail and the response after consumption. Here the results of the using the dose response model of Powell et al. (2000) instead of the model based on the Japanese outbreak (chapter 4) suggests that the selection of the dose response model has a large impact on the risk estimate. The uncertainty around the estimated dose response model parameter is large, and the Japanese outbreak caused by a very virulent strain of STEC O157 in a school lunch

may not be representative for Europeans eating steak tartare. This suggests that improved dose response modelling is crucial.

7.1.3 Scenario analysis and the statement of purpose

The scenario analysis has been set up to study the effect of quantified uncertainties and some model assumptions on the baseline model results. It was found that uncertainty parameters in the top of the food pathway had large impact on the result: Animal prevalence at slaughter, STEC concentration in faeces and carcass contamination could not be determined precisely and yet taking reasonable alternatives gave very different risk estimates. Also, using an alternative dose response model may have a large impact. If the (more realistic but also more uncertain) hypergeometric model is used instead of the exponential model, fitting the same Japanese outbreak data, the estimated number of cases is almost doubled. If the dose response envelope of Powell et al. (2000) is used, the most likely risk estimate is extremely low. The uncertainty in consumer behaviour and the effects of preparation also contribute significantly to the total uncertainty.

Although the risk estimate from the risk model is quite uncertain, the risk assessment performed is useful. Not only has it been a good and instructive exercise in risk assessment modelling, covering the whole trajectory from 'farm to case', it also identifies important and less important gaps of knowledge, and allows a provisional insight in comparison of intervention strategies for risk management. Based on present knowledge and expertise the model suggests that incidence in the Netherlands due to steak tartare consumption may be lowered most by lowering the prevalence and concentration of STEC O157 in cattle (model parameters P_f and C_{ij}), by improved hygiene at slaughter (model parameters a and G_{prc}) or by an increased frequency of industrial processing. Product control (and monitoring) at retail level seems rather useless, due to the predicted low prevalence and low concentration. Growth of STEC O157 during storage is unlikely. An information campaign for consumers promoting better cooking of the tartare may be helpful, but as the effect of such a campaign is very uncertain, well cooking is not in line with the Dutch tradition of eating 'red' tartare and eating 'well done' steak tartare is not very tasteful, the public health effect of such an action is doubtful. The campaign might of course result in a decrease of the total consumption of steak tartare. There is no doubt that this would be effective.

Revisiting the statement of purpose as described in chapter 2, shows that we have not exactly met the purposes set. The first purpose was to derive the exposure distribution to STEC O157 in steak tartare for consumers in the Netherlands. The exposure distribution as illustrated in Fig 5-1 comes close, but is the exposure if all tartare patties would be consumed raw. This distribution is preferred for practical reasons: The @Risk spreadsheet had to be split in two, and the point in the food pathway before preparation was the preferable point of splitting.

The second purpose was to assess the health burden. We estimated the number of cases due to STEC O157 in steak tartare in the Netherlands among people of one year of age and older. The health burden was not investigated further by e.g. weighing cases of gastro enteritis with

bloody/non bloody diarrhoea and HUS. This is planned in the near future (Havelaar et al, *unpublished*). Finally, the last purpose was to assess the effects of intervention measures: ‘logistic slaughter’ and ‘information campaign’. This has not been done. Unfortunately, data collection, gathering of information on the food pathway and production processes, the construction of the risk model and the risk assessment took much more time than planned, and did not allow focussing narrower on these two measures. Of course, this is rather unfortunate, as selection of these measures and the research on information campaigns were quite an exercise on their own (see section 2.5 and chapter 6). However, the model allowed a rough comparison of intervention at different stages in the food pathway. It appears that intervention at slaughter is preferred, although such an intervention needs further study on details and feasibility. As storage does not seem to affect the final risk and the possibilities of changing consumer habits concerning cooking and consumption of steak tartare are doubtful, an information campaign aiming at better storage and preparation, as suggested by the Netherlands Nutrition Centre (section 6.3.), will probably not reduce the risk of STEC O157 infection as a consequence of steak tartare consumption.

7.2 The risk model

7.2.1 The food pathway

The risk model described in this report consists of an exposure assessment model and an effect model. For the exposure model, the Modular Process Risk Model (MPRM) methodology has been applied. After a precise definition of the food pathway to be modelled, model equations and parameters for the basic process models are defined for each step in the process.

The food pathway model, as illustrated in Figure 3-6, is a strong simplification of the complex food pathway, as illustrated in Figures 3-1 and 3-2. Simplification is required for modelling, and is always disputable. As in any model, the features that are considered essential for the research objective are included and those that are considered irrelevant are omitted. In the simplifications applied here, the focus has been on the route of transmission of the hazard. The main routes of transmission are considered ‘standard’, and only routes that are expected to give significantly different risk estimates are considered separately. We thus differentiated three routes of exposure, characterised by the size of the slaughterhouse (and thus the number of animals slaughtered daily) and the size of the ground beef batches produced. These characteristics were expected to have a significant impact on the risk estimate. The scenario analysis shows that, in part, this is true.

In the food pathway model several aspects were not considered, for example a differentiation between dairy cattle, veal cattle and veal calves, foreign and Dutch animals, seasonal effects, restaurants and private households. From information from the Product Boards (PVE 2001), for example, it can be derived that in 1998 61% of Dutch consumption of cattle meat is

imported meat (products) (see section 3.1.3). For steak tartare this percentage is unknown, but might be lower (see section 3.2.4.2). In general, the obtained information on the origin of the meat used for steak tartare is too ambiguous to include the differences in the model. Besides, data that allow a difference between parameter estimates for different sources of meat are lacking. The same arguments hold for the difference between restaurants and private households: processing information is not sufficiently documented to differentiate the two in the risk model. Seasonal effects may be important, as the prevalence of STEC O157 in farm animals is variable with the season (see Appendix 2). To incorporate those effects we used a prevalence estimate of positive animals at slaughter, P_f , based on a year round studies on dairy cattle (see section 3.2.1)

7.2.2 Process step models

In the models for the different process steps along the food pathway, we used a variety of assumptions in modelling and for estimation of parameters, which are discussed below.

In *Step 1*, the carcass contamination, it is assumed that each carcass is contaminated with animal faeces. According to the expert panel (*pers. comm*) this was a reasonable assumption. The assumed (variability distribution of the) concentration of STEC O157 in cattle faeces is based on one North American study among calves, as used by Cassin et al. (1998). This is therefore a rather uncertain estimate for the Dutch situation among cattle at the moment of slaughter. However, other applicable data are not known to us. The faecal carcass contamination (expressed as gram faeces per carcass) is an important characteristic in the model, with apparently a large impact on the model outcome. Applicable data are lacking here, although a translation of North American data is possible as outlined in 3.3.4.2. We used the expert panel estimate, which lies close to the translated North American data. Clearly, quantitative microbiological research on carcass contamination at slaughter, focussing on the needs of risk assessment, is urgently needed.

We modelled carcass contamination by assuming that several animals may contaminate a carcass (by whatever route), and by applying the methodology explained in Appendix 3, which incorporates variability in relative contributions of animals. The effect on the risk estimate of using this rather complex methodology is small in the case of the present risk assessment study. This is shown by the $b_1=b_5=100$ alternative scenario, which represents (almost) equal relative contributions (see Table 5-8). This does however not mean that the methodology may not be worthwhile to apply successfully in other contexts.

In *Step 2* carcass halving is modelled, with incorporation of a potential clustering effect. The expert panel doubted the importance of incorporation of this process in the model, which, regarding the effect of clustering on the modelling results (Table 5-8) is not incorrect. In the risk model only one carcass half is considered. This is done to simplify the modelling. The effect of this assumption to the end result is unknown, but presumably small.

As in the study of Cassin et al. (1998), a factor describing growth or inactivation of STEC O157 on the carcass surface during storage in the slaughterhouse (G_{prc}) is included in *Step 3*

of the model. As shown in the scenario analysis, this parameter has a relatively large impact on the final risk estimate. The minimum, most likely and maximum value as estimated by the expert panel are implemented in a Triangle distribution to allow comparison with that of Cassin et al. (1998). As for the faecal contamination parameters, the parameter estimates are merely (educated) guesses, and quantitative research is necessary.

In *Step 4* partitioning of trimmings is modelled according to the partitioning model methodology described in this report (3.3.2.2.). The variability distribution of the weights of the trimmings (that is of the total weight of all the trimmings from one carcass used for a ground beef batch) is derived from the distribution of ground beef batch weights, carcass weights and the number of animals contributing meat to the ground beef batch. This distribution can probably be checked with data, but these were not available to us. In the analysis it is assumed that the probability of contamination with STEC O157 on meat destined for steak tartare is equal to the probability of contamination of the 'average meat' from the carcass. As steak tartare meat is taken from specific parts of the carcass (see 3.2.4) this assumption may be wrong. However, we had no other information on differences in contamination levels than qualitative guesses, which could not be incorporated into the model in a meaningful way. If required, this effect may be incorporated by lowering for example the concentration in the faeces (C_{ij}) by a fixed or variable factor.

In *Step 5* the ground beef batch production is modelled as a mixing process. As the weight of the ground beef batch is probably correlated to the number of animals contributing to it, a correlation is incorporated which proves to have little effect on the risk estimate.

In *Step 6* tartare patty production is modelled. It is implicitly assumed that patties are made before storage, which need not be the case. It might therefore be that partitioning and growth are modelled in the wrong order. In general (and especially when growth is as unimportant as it is here), this will presumably have little or no impact on the final risk estimate. The clustering factor used here, need not only represent the cell clustering on microscopic scale, but also the effect that a large (e.g. 1000 kg) batch will not be well mixed. This is the reason why we assumed quite some clustering in the baseline model, although some experimental studies suggest otherwise (Reinders et al. 2001). The effect of this clustering on the risk estimate is relatively small.

In *Step 7* growth during storage is modelled. The storage period encompasses the whole period from steak tartare production to consumption, and therefore includes both retail and household storage. The storage time and temperature distributions used here are derived from household data. It holds the (reasonable) assumption that steak tartare meat is stored below 4°C in professional settings. The growth model used has been derived from PMP data (USDA 1998). Data and models on growth of STEC O157 from other sources (e.g. Nauta and Dufrenne 1999) could not easily be applied, as they do not incorporate secondary models of generation time and lag phase duration. In general, applying predictive microbiology models in risk assessment is not straightforward (Nauta 2001a), as relevant variability in lag phase duration and growth rate are generally not included. However, alternatives are not available. The PMP model is well documented and widely used. As the growth model predicts hardly any growth under the conditions modelled, the use of other models is not considered.

Inactivation during preparation is modelled in *Step 8*. Due to a lack of data we use a simple model. Only three preparation styles (raw, medium, well done) are modelled, where a continuum of preparation styles may be realistic. The frequency in which each of the three preparation styles occurs is based on data that are insufficiently documented for our purposes (Kistemaker et al. 1998) and uncertain expert estimates. This was, however, the best we could get. Literature on STEC O157 inactivation models is not used, because temperature profiles in the steak tartare patty in realistic daily household situations are not known. The inactivation factors f_{survive} are estimated by the expert panel on the basis of prior knowledge of the results of Jackson et al. (1996). As the effect of inactivation may be important for risk mitigation, it can be concluded that consumer behaviour at this point urgently needs further quantitative research.

In *Step 9* the exposure at consumption is calculated at population level. Here the third Dutch nutrition surveillance (Kistemaker et al. 1998) proved very useful, but the low frequency of consumption of raw steak tartare made it difficult to estimate its frequency of consumption. As the effect on the risk estimate of using alternative methods to estimate this frequency was small, this problem was not important in this particular risk assessment.

7.2.3 Parameter estimation by expert elicitation

Many of the parameter values are estimated by an expert panel during the expert workshop held at RIVM (see 3.3.4.1). The use of expert judgement for parameter estimation and uncertainty analysis is a field of research on its own (e.g. Morgan and Henrion 1990, Meyer and Booker 1991, Cooke 1991), which is relatively new to the authors. Time did not allow for the organisation of a thoroughly prepared expert elicitation workshop in line with the guidelines in this field. The reason for organising one plenary expert elicitation session was mainly pragmatic. An alternative would have been to visit all experts separately, which would allow a specific questioning on the experts expertise, and a better control on the way that questions of the risk assessors are interpreted by the expert. We now had a meeting with a variety of people (with background in animal health, food microbiology, agricultural science, meat technology etc.) who answered questions on aspects of the whole food pathway. Although all areas of the pathway were (more or less) covered, and expertise was weighted in the analysis, this may not have yielded the optimal result. It served however the main objective of the risk assessment project, that is to obtain experience of the risk assessment process, with its potential pitfalls.

In general, expert opinion is not an ideal way to estimate parameter values. Estimates may be biased and experts may influence each other. (Scientific) data are always preferable. However, if data are not available expert elicitation is a last resort to allow for risk assessment after all. Risk assessment is applied for risk management as a tool to evaluate risk mitigation strategies. If the public demands intervention in the short term, decisions cannot await outcome of thorough research. Risk managers need to come to decisions on the basis of the available information, and risk assessment offers a methodology to consistently order this

information. When decisions are to be taken on the basis of expert judgement, the best option is to do it via risk assessment.

7.2.4 The effect model

The careful investigation of the STEC O157 outbreak in Japan (chapter 4) has enabled us to estimate the relationship between ingested dose of the pathogen and the probability of infection. To our knowledge (at the end of the year 2000), this is the only STEC O157 outbreak study that documents the dose, the number of people exposed, as well as those whose stools were tested positive for STEC O157. As investigators hopefully increasingly will follow this good practice, we expect that in the future similar information will become available.

A disadvantage of having data of only one outbreak is that more than one scenario may equally well explain the outbreak. On the basis of the reported data alone it is not possible to explain why 620 of the 828 children who had consumed the contaminated school lunch became infected while the remaining 208 children did not. One may expect that all children would have been infected if they ingested a high enough dose of STEC O157. Alternatively, however, one also may argue that those 208 children would never have been infected because they were completely resistant to colonisation by the pathogen.

The previous reports by Haas et al. (2000) and Powell et al. (2000) underestimated the risk associated with STEC O157. To be fair, our case definition for a positive response does differ from those previous studies, i.e. faecal excretion as opposed to symptoms. So the apparent difference may be considered an artefact due to different case definitions. This is, however, not the case. The Japanese outbreak data show that approximately 55% of those whose stools were tested positive for STEC O157 did develop symptoms. If we take this into consideration, the number of symptomatic people would be about half that of faecal positives. From the figure comparing several dose-response models (Figure 4-1), it is easy to see that, even if we adjust our case definition, the results of Haas et al. (2000) and Powell et al. (2000) would still produce much lower estimates of the risk of STEC O157. The use of non-human host species (rabbit) and surrogate pathogens (EPEC and *Shigella dysenteriae*) to approximate STEC O157 infection in human better accounts for the fact that the previous attempts failed to be in line with the outbreak observation.

An important question that we did not investigate in this report is how well the estimated exposure approximates the ingested dose. When integrating the exposure module to the dose-response module, we implicitly assume that all STEC O157 in tartare are indeed as virulent as the outbreak isolates. This assumption is however, open to a further discussion. A recent research in Denmark (B. Christensen, *pers. comm.*) demonstrated that subtypes STX2 and STX2vha in combination were the most common clinical isolates, while they were rarely isolated from cattle. Incidentally, the isolate of the Japanese outbreak was tested positive for STX2. This raises a possibility that only a small fraction of STEC O157 in tartare is indeed so virulent as to cause illness, and a great majority of the pathogen is less virulent. If this were

indeed true, using exposure by all STEC O157 in tartare would have been an overestimate for ingested infectious dose. This may be a part of the reason why our estimated STEC O157 cases in the Netherlands due to a consumption of tartare alone exceeds the epidemiological data concerning all food-related STEC O157 cases (Havelaar et al. 2001).

7.3 Lessons on risk assessment modelling

As stated in chapter 1, the main objective of this risk assessment study was to gain experience in conducting a risk assessment ‘from farm to fork and beyond’ and discover potential pitfalls. This objective is achieved. Some of our experiences that may be important for future quantitative microbiological risk assessments are discussed below.

First, the food pathway complexity complicates risk assessment. Typically, microbiological risk assessment focuses on some specific processes that are considered characteristic for the production sector. For slaughter, one models one slaughterhouse with one type of slaughtering practice. This is not problematic as long as this practice is a good model for slaughterhouses considered relevant for the risk assessment. However, like in the Dutch situation, a variety of slaughterhouses may exist, with a varying number of animals slaughtered per day and variable levels of hygiene. Regulations may be different for different sizes of slaughterhouses and for slaughterhouses that produce meat destined for export or not. We tried to incorporate the essential differences between the slaughterhouses in the model by separating three different routes of exposure, characterised by some model parameter differences that we thought to be relevant. However, some aspects may be overlooked. Import of cattle, carcasses and meat complicates this even further.

As software tool for the risk model we used an Excel spreadsheet with @Risk as an add on. The use of a spreadsheet is simple and gives a good overview of the data and modelling steps. The @Risk program is built for risk assessment modelling and has the advantage of many useful interfaces and (e.g.) graphical facilities. However, for large and complex risk assessment models the @Risk model may become too large. Simulations take a very long time, or may even fail. A problem here is that, with the low prevalence of positive units, many iterations of the Monte Carlo model are necessary. We tried to circumvent this problem as much as possible for example by splitting the risk model in two, but the ‘zero’ results remained problematic. Even after doing 50000 iterations of a complex model, model results may still be based on relatively few positive (non-zero) modelling results (that is about a few hundred). Doing ‘useless’ calculations is not easily prevented in @Risk, but it may be by using an other programming language.

The scarcity of data is evident. Comparison of the data gathered given in section 3.1 and 3.2 and the data used in the risk model described in section 3.3 shows there is very little overlap. It means that very little of the available data is useful for risk assessment. This is caused by the fact that data are not quantitative or not representative for the food pathway and the population modelled and are centred in a few traditional areas of research. There are for example quite some data on prevalence of STEC O157 at farm level or seasonal fluctuations,

but these could not be implemented in the risk model. There are many data on thermal inactivation, but not in 'household situations' relevant for risk assessment. Quantification of numbers of bacteria is difficult and time consuming, and therefore rarely done.

The scarcity of data may be in conflict with the complexity of the model applied. A correct model estimate depends on the combination of a good model and sufficient data. Applying a model that includes subtleties (like all kinds of variability or cell clustering) without proper data does not improve the value of the estimate. Using complex models may therefore seem an unnecessary complicating exercise. However, developing complex models falls in the scope of the risk assessment project to develop tools for risk assessment in general. Also, more complex models may not give better risk estimates, but they do allow a study for the effect of things like variability and clustering. This has a value on its own that should not be overlooked: It illustrates the effects of model assumptions and points at gaps in data and knowledge that may be (ir-)relevant for risk assessments.

An important characteristic of the risk model described in this report is that uncertainty is not (quantitatively) included. In general, modelling uncertainty is considered one of the most important reasons for applying Monte Carlo simulations. Estimating uncertainty attending risk estimates is prescribed in the definition of 'Risk characterisation' in an official risk assessment (CODEX Alimentarius Commission 1998). This is for a good reason: if a public health risk is estimated and communicated without an uncertainty interval this may suggest unrealistic confidence in the estimate and lead to invalid conclusions of risk managers, stakeholders or the public. Nonetheless, quantifying uncertainty was not possible in the risk assessment study presented here. After making a clear distinction between variability and uncertainty, it was decided to include only variability in the model. Second order Monte Carlo modelling would have been very complicating, but could never have included all uncertainties present. If variability and uncertainty are not neatly separated, this may result in an improper uncertainty estimate, and even a false estimate of the risk (Nauta 2000).

The STEC O157 risk assessment described in this report is not the first risk assessment on this pathogen in a beef product. Other (un-)published studies are that of Cassin et al. (1998), Marks et al. (1998) and Coleman et al. (1998). The study of Cassin et al. (1998) on *E. coli* O157 in hamburger has been mentioned before. Several of their model assumptions and parameter estimates have been applied in this study, or have been used as alternative in the scenario analysis. Important differences with this study are not only the product and the country of interest, but also the use of the Modular Process Risk Model (MPRM). Actually, Cassin et al. (1998) introduced the Process Risk Model. Main differences in our approach are the modular structure, basic process definition, consistent separation of uncertainty and variability, the incorporation of potential cell clustering, the definition of units and the use of numbers of pathogens per unit instead of concentrations (thus preventing calculations with fractions of cells).

As before (Nauta 2001b), the MPRM methodology proves useful as a tool to structure the risk model. Focussing on the food pathway, it clearly defined the process, product and population of interest in the risk assessment. Gaps in data and knowledge are easily identified. The use of the 'basic process' approach simplifies the construction of the food pathway model

structure. Still, in the risk assessment study of STEC O157 in steak tartare it was found that especially in the first step of the process the 'basic process' structure is difficult to implement. Here some 'ad hoc' models on carcass contamination had to be built. Probably this is inevitable when complex processes are to be modelled in more detail. However, with further use of the 'basic modelling' approach typical for MPRM, a library of basic process models may be build, which in the future will facilitate quantitative risk assessment.

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Appendix 2 Data

2.1 Prevalence at the Farm

2.1.F1

Heuvelink et al. 1998b

Heuvelink 2000d

Summary

Serotype

STEC O157, basis type for article was also STEC O157.

Prevalence

7 of 10 farms positive.

proportion of infected animals 0.8 to 22.4 %.

Table A2-1 Prevalence of STEC O157 at animal level per farm.

Farm no.	No. of animals positive	Total no. of animals	Percentage
1	27	140	19.3
2	15	67	22.4
3	7	112	6.3
4	3	162	1.9
5	17	83	20.5
6	1	120	0.8
7	5	100	5.0
8	0	100	0.0
9	0	195	0.0
10	0	73	0.0
Total	75	1152	6.5

Virulence-associated genes

All are VT2 and eae positive.

Detection method

Enrichment in mEC+n followed by IMS and isolation on CT-SMAC.

Sampling material

faecal samples (digital rectal retrieval).

Sampling strategy

Samples from all cattle present, approx. 50 g each.

Sampling location

Prevalence study: ten Dutch dairy farms . Five farms that delivered positive (farm 1 to 5) and five farms that delivered negative (farm 6 to 10) cattle for slaughter in a previous study (August 1996). Follow-up study: two positive and two negative dairy farms.

Period of sampling

Prevalence study: September 1996 through November 1996.

Follow-up study: September 1996 through November 1997

Age

Excretion rate highest in calves ages 4 to 12 months in the prevalence study (21.2%) and in the follow-up study (11.8%). This is confirmed by GEE analysis.

Time dynamics

Two positive and two negative farms revisited five times at intervals of 3 months. Each farm was tested positive at least once. The proportion of infected cattle varied from 0 to 61 % (follow-up study). Excretion rates peaked in summer and were lowest in winter (statistically significant, confirmed by GEE analysis). 14 of 18 samples from fresh cowpats were positive in farm 10.

*Details**Age*

Table A2-2 Prevalence of STEC O157 at animal level per age category (Prevalence study).

Age	No. animals positive	Total no. of animals	Percentage
< 4 months	4	60	6.7
4-12 months	29	137	21.2
1-2 years	5	135	3.7
2-3 years	3	134	2.2
> 3 years	34	318	10.7

Table A2-3 Prevalence of STEC O157 at animal level per age category (Follow-up study).

Age	No. animals positive	Total no. of animals
< 4 months	8	131
4-12 months	36	306
1-2 years	13	265
2-3 years	11	340
> 3 years	41	824

*Time dynamics***Table A2-4** Prevalence of STEC O157 at animal level per farm and date (Follow-up study).

Farm	Date	No. of cattle positive	Total no. of cattle	Percentage
2	Sep 96	15	67	22.4
	Dec 96	8	106	7.5
	Mar 97	0	99	0.0
	Jun 97	0	99	0.0
	Sep 97	36	59	61.0
	Nov 97	15	104	14.4
5	Oct 96	17	83	20.5
	Jan 97	1	83	1.2
	Apr 97	2	79	2.5
	Jun 97	9	52	17.3
	Sep 97	2	38	5.3
	Nov 97	0	41	0.0
8	Nov 96	0	100	0.0
	Jan 97	0	129	0.0
	Apr 97	1	131	0.8
	Jul 97	0	99	0.0
	Sep 97	0	81	0.0

	Nov 97	0	110	0.0
10	Nov 96	0	73	0.0
	Feb 97	0	73	0.0
	Apr 97	0	64	0.0
	Sep 97	2	22	9.1
	Nov 97	1	74	1.4

Additional information:

- 86, 58, 82, 109, 104, 77 cattle were sampled 1, 2, 3, 4, 5, 6 times.
- 93 cattle were tested positive: 78, 14, 1 had 1, 2, 3 positive samples.
- Longest period of excretion was approx. 3 months, but most negative within 3 months.
- Individual cattle could originally be identified via ear tag numbers.

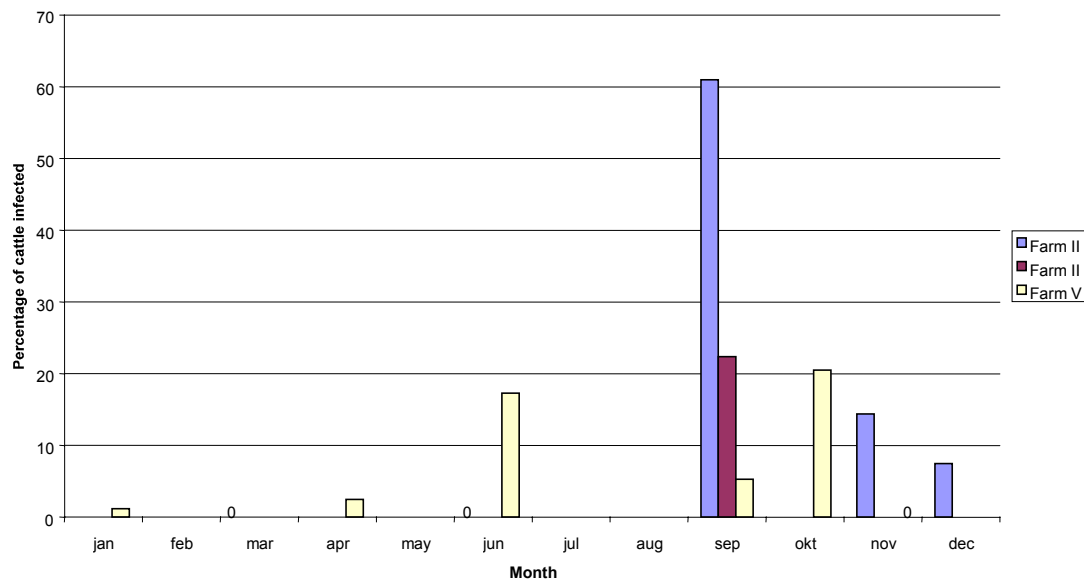


Figure A2-1 Graphical representation of the results for farm 2 and 5 of Table A2-4.

Table A2-5 Prevalence of STEC O157 at animal level per period (Follow-up study), summary of Table A2-4.

Period	No. of animals positive	Total no. of animals
Jan-Mar	1	384
Apr-Jun	12	425
Jul-Sep	55	366
Oct-Dec	41	691

Interpretation

A difference between immature and adult can be caused by:

- 1) rumen function
- 2) diet
- 3) immune response
- 4) cattle management

A season effect can be caused by:

- 1) indoor/pasture
- 2) diet

2.1.F2

Reinders and Bijker 1999

Summary

The part of this study on veal bulls in the slaughterhouse is presented under the section 2.2 'Slaughterhouse'.

Serotype

STEC O157, base for report was also STEC O157.

Prevalence

Screening

2 of 34 farms were positive. One of these farms (L) was visited twice (June and November 1997) and was positive twice.

Longitudinal study

Farm L:

March 1998: 7 positive of 70 samples;

29 June: one dubious (positive or negative) case of 70 samples;
and another two dubious cases.

Farm M:

always positive except once.

Farm M was found positive previously in '95 or '96 by the slaughterhouse study of Heuvelink et al. 1998a and Heuvelink 2000g (pers.comm. Reinders, 20 April 2000).

Virulence-associated genes

Testing for VT1, VT2, eae with PCR and for EHEC-plasmide.

Detection method

Enrichment in mTSBa; IMS; isolation on CT-SMAC.

Sampling material

Screening

faeces (rectal retrieval) from dairy cattle.

Longitudinal

swabs from dairy cattle.

Sampling strategy

Screening

The research population was the set of farms used by the Faculty of Veterinary Medicine. 20 faeces samples were taken per farm. 37 investigations were done on 34 farms. For the first 24 investigations, samples were pooled by four. Later on swab samples were taken that were not pooled.

Longitudinal study

Two contaminated farms from the screening study, two weekly sampling of all cattle.

Sampling location

Dairy cattle farms.

Period of sampling

Screening

January to December 1997.

Longitudinal study

March to October 1998 (farm L); May to October 1998 (farm M).

*Herd size*Longitudinal study

Farm L has ca. 70 cattle.

Farm M has ca. 81 cattle (derived from page 24: 31 % is 25 animals).

*Geographic distribution*Longitudinal study

Limited information on location of farms.

*Time dynamics*Longitudinal study

Farm L: see 'prevalence'.

Farm M: see 'details'.

Details*Time dynamics*

Table A2-6 No. of STEC O157-positive animals related to sampling day for Farm M. Estimation based on Figure 2 on page 23.

Day	No. of positive animals
0	0
14	2
28	9
42	5
56	12
70	16
84	17
94	25
112	8
126	9
140	6

Table A2-7 Frequency distribution of the number of STEC O157-positive samples per individual animal for Farm M. Estimation based on Figure 2/3 on page 25.

No. of positive samples	No. of animals
0	36
1	22
2	6
3	4
4	10
5	1
6	1
7	0
8	0
9	1

Of 36 calves and yearlings that were present in the young cattle stable during the whole investigation, 31 were one or more times positive.

Suppliers of positive veal bulls

Data from the positive supplier to Slaughterhouse no.1 (June 1997): it had 49 dairy cattle, 20 young cattle and 23 veal bulls. 4 of 25 dairy cattle, 0 of 6 young cattle and 4 of 11 veal bulls were positive.

Table A2-8 Data from the 2 positive suppliers to Slaughterhouse no. 2 of November 1997.

Supplier	January 1998		March 1998	
	No. of samples	No. positive samples	No. of samples	No. positive samples
A	16	2	31	3
B	19	6	40	8

Supplier A had 31 veal bulls. Supplier B had ca 150 veal bulls.

2.1.F3

Heuvelink et al. 1999b

Summary

Serotype

STEC O157, basis type for report was E. coli O157.

*Prevalence***Table A2-9** Prevalence of STEC O157 at farm level (Page 17 Table 5).

Type	No. of positive herds	No. of sampled herds	Percentage	90% conf.int.
Veal calves	3	191	1.6	0.0 – 3.0
Dairy cattle	5	136	3.7	

Virulence-associated genes

One of the O157-positive dairy cattle samples proved to be negative for VT1, VT2 and eae (Page 17 Table 6).

Detection method

Enrichment in mTSB+a followed by IMS and isolation on CT-SMAC.

Sampling material

faeces from the floor.

*Sampling strategy*General

The number of sampled herds and the number of sampled animals within a herd (both herd size-dependent) was based on statistical considerations. Within stratification for farms size, farms were selected at random. Samples (1-60) were pooled to 2 to 5 samples (also herd size-dependent) of approx. 100 g each and then pooled to one sample.

Veal calves

192 herds were sampled, with in total 845 pooled faeces samples.

Dairy cattle

136 herds were sampled, with in total 571 pooled faeces samples.

Sampling location

farm.

Period of sampling

April 1997 to March 1998 inclusive.

*Herd size*Veal calves

Sampled herd size distribution: Page 12 Figure 1C.

Dairy cattle

Sampled herd size distribution: Page 12 Figure 1D.

Age

Veal calves

Sampled age distribution: Page 13 Figure 2C. Mostly (or exclusively) veal calf herds > 18 weeks were positive (Page 33 Appendix 3 Figure 3).

Dairy cattle

Herds have animals with varying age.

Geographic distribution

The geographic distribution of veal calf farms (A) and sampled veal calf farms (B): Page 31 Appendix 2C.

The geographic distribution of dairy cattle farms (A) and sampled dairy cattle farms (B): Page 32 Appendix 2D.

Time dynamics

Veal calves only positive in the second quarter, dairy cattle positive in the third and fourth quarter (Page 17 Figure 5 and page 34 Appendix 4).

Details

Prevalence

2444 veal calf herds and 1.6 % positive means that 38 veal calf herds are positive;
37465 dairy herds and 3.7 % positive means that 1377 dairy cattle herds are positive.

Detection method

- enrichment of 10 g faeces in 90 ml modified tryptone soy broth containing acriflavin (mTSB + a);
- immunomagnetic separation using 1 ml of this enrichment culture, in duplicate;
- inoculation onto sorbitol MacConkey agar supplemented with cefixime and potassium tellurite (CT-SMAC)
- confirmation by inoculation onto eosin methylene blue (EMB) and onto SMAC supplemented with 4-methylumbelliferyl- β -D-glucuronide (SMAC-MUG);
- testing for agglutination with an E. coli O157 latex test kit;
- serotyping by LIS;
- determination of the presence of VT1, VT2, and eae gene sequences by a multiplex PCR assay.

*Sampling strategy*General**Table A2-10** No. of samples per herd and pooling strategy as related to herd size.

Herd size	Number of samples	Number of pooled samples
1-24	equal to number of animals to a maximum of 20	2
25-29	20	2
30-39	25	2
40-49	30	3
50-59	35	3
60-89	40	4
90-199	50	4
200-499	55	5
≥500	60	5

NB for E. coli O158 all pooled samples were pooled to one sample (p. 10).

Veal calves**Table A2-11** No. of samples and planned number of samples as related to farm size for veal calves (Page 27 Appendix 1C).

Farm size	Number	Planned number to sample
0-100	596	11
100-300	703	47
300-500	521	70
500-1000	549	111
>1000	75	32
Total	2444	271

Dairy cattle**Table A2-12** No. of samples and planned number of samples as related to farm size for dairy cattle (Page 28 Appendix 1D).

Farm size	Number	Planned number to sample
1-10	3380	1
10-20	3354	3
20-30	4621	6
30-50	11269	24
50-100	13172	53
>100	1669	11
Total	37465	98

Time dynamics

In the table below, one of the positive dairy cattle herds is *E. coli* O157 positive and not STEC O157-positive. Which one this is, might be retrievable from the original data.

Table A2-13 Prevalence of STEC O157 at farm level as related to quarter of the year.

Type	Quarter	No. of positive herds	No. of sampled herds	Percentage (%)
Dairy cattle	II 97	0	18	0
	III 97	2	25	8.0
	IV 97	4	39	10.3
	I 98	0	54	0
Veal calves	II 97	3	24	12.5
	III 97	0	23	0
	IV 97	0	66	0
	I 98	0	78	0

2.1.F4

Van de Giessen 1998

Summary*Serotype*

STEC O157 (pers.comm. Cecile Deisz, 11 April 2000).

*Prevalence***Table A2-14** Prevalence of STEC O157 at farm level in 1998.

Type	No. of positive herds	No. of sampled herds	Percentage (%)
Dairy cattle	13	267	4.9
Veal calves	8	152	5.3

*Sampling strategy*General

The number of sampled herds and the number of sampled animals within a herd (both herd size-dependent) was based on statistical considerations. Within stratification for farms size, farms were selected at random. Samples (1-60) were pooled to 2 to 5 samples (also herd size-dependent) of approx. 100 g each and then pooled to one sample.

Period of sampling

1998

Time dynamics

Data broken down to quarters in 'Details'.

Details*Time dynamics***Table A2-15** Prevalence of STEC O157 at farm level in 1998 related to quarter of the year

Type	Quarter	No. of positive herds	No. of sampled herds	Percentage (%)
Dairy cattle	I	0	54	0
	II	3	68	4.4
	III	3	69	4.3
	IV	7	76	9.2
Veal calves	I	0	78	0
	II	2	39	5.1
	III	1	10	10.0
	IV	5	25	20

2.1.F5

Van de Giessen 1999

Summary*Serotype*

STEC O157 (pers. comm. Cecile Deisz 11 April 2000).

*Prevalence***Table A2-16** Prevalence of STEC O157 at farm level in 1999.

Type	No. of positive herds	No. of sampled herds	Percentage (%)
Dairy cattle	13	161	8.1
Veal calves	8	60	13.3

*Sampling strategy*General

The number of sampled herds and the number of sampled animals within a herd (both herd size-dependent) was based on statistical considerations. Within stratification for farms size, farms were selected at random. Samples (1-60) were pooled to 2 to 5 samples (also herd size-dependent) of approx. 100 g each and then pooled to one sample.

Period of sampling

1999

Time dynamics

Data broken down to quarters in 'Details'.

Details*Time dynamics***Table A2-17** Prevalence of STEC O157 at farm level in 1999 related to quarter of the year

Type	Quarter	No. of positive herds	No. of sampled herds	Percentage (%)
Dairy cattle	I	0	46	0
	II	1	42	2.4
	III	7	47	14.9
	IV	5	26	19.2
Veal calves	I	2	28	7.1
	II	1	4	25.0
	III	3	13	23.1
	IV	2	15	13.3

2.1.F6

Van de Giessen 2000

Summary*Serotype*

E. coli O157; testing for VT1, VT2 and eae genes is still to be done (e-mail W.D.C. Dam-Deisz, 4-7-00).

*Prevalence***Table A2-18** Prevalence of STEC O157 at farm level in 2000

Type	No. of positive herds	No. of sampled herds	Percentage (%)
Dairy cattle	1	55	1.8
Veal calves	1	32	3.1

*Sampling strategy*General

The number of sampled herds and the number of sampled animals within a herd (both herd size-dependent) was based on statistical considerations. Within stratification for farms size, farms were selected at random. Samples (1-60) were pooled to 2 to 5 samples (also herd size-dependent) of approx. 100 g each and then pooled to one sample.

Period of sampling

Jan – March 2000

Time dynamics

Data broken down to quarters in 'Details'.

Details*Time dynamics*

Table A2-19 Prevalence of STEC O157 at farm level in 2000 related to quarter of the year

Type	Quarter	No. of positive herds	No. of sampled herds	Percentage (%)
Dairy cattle	I	1	55	1.8
Veal calves	I	1	32	3.1

Below, the data from Van de Giessen 1998, Van de Giessen 1999, Heuvelink et al. 1999b, Van de Giessen 2000 are combined. For dairy cattle, there seems to be a relationship between the quarter of the year and the percentage positive herds (Fig A2-2 and A2-3). The level in the third and fourth quarter seems to be related to the year. For veal calves, the only observation is that the level is consistently low in the first quarter, whereas in the other quarters mean and variance are higher (Fig. A2-4 and A2-5).

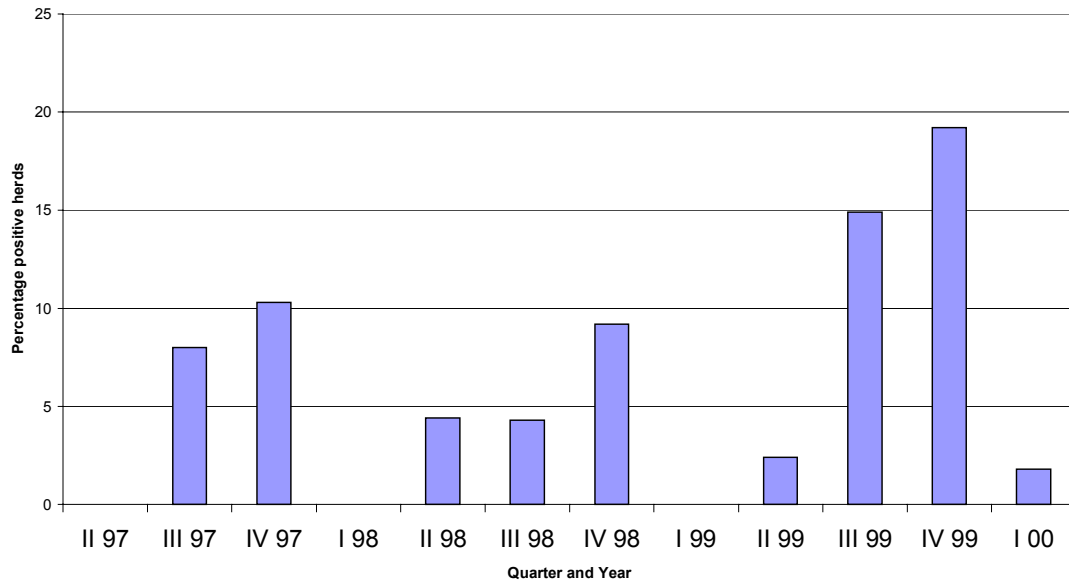


Fig. A2-2 STEC O157 surveillance data in dairy cattle. Relationship with quarter of the year.

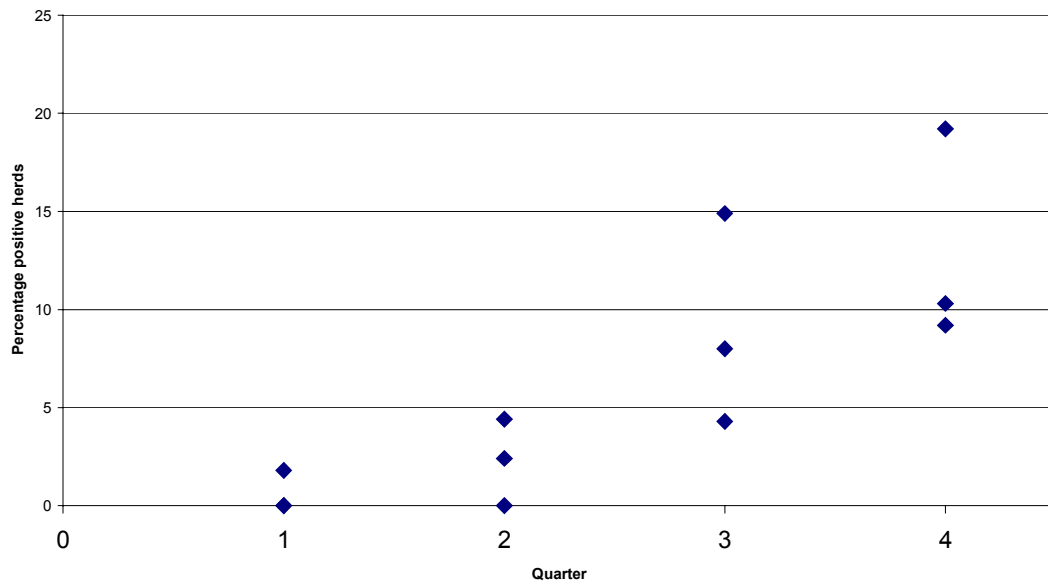


Fig. A2-3 STEC O157 surveillance data in dairy cattle. Relationship with quarter of the year, aggregated over the years.

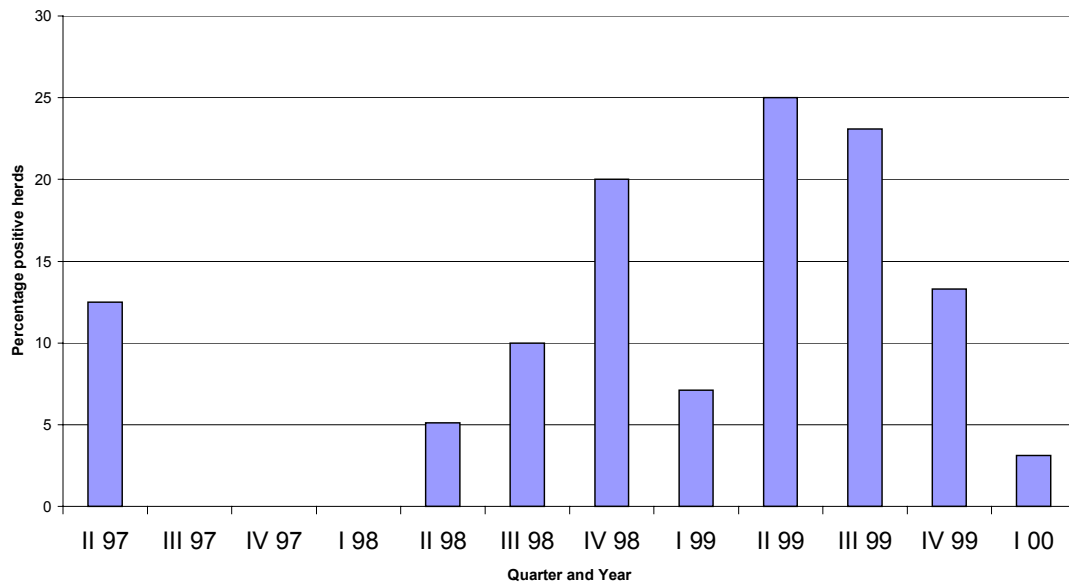


Fig. A2-4 STEC O157 surveillance data in veal calves. Relationship with quarter of the year.

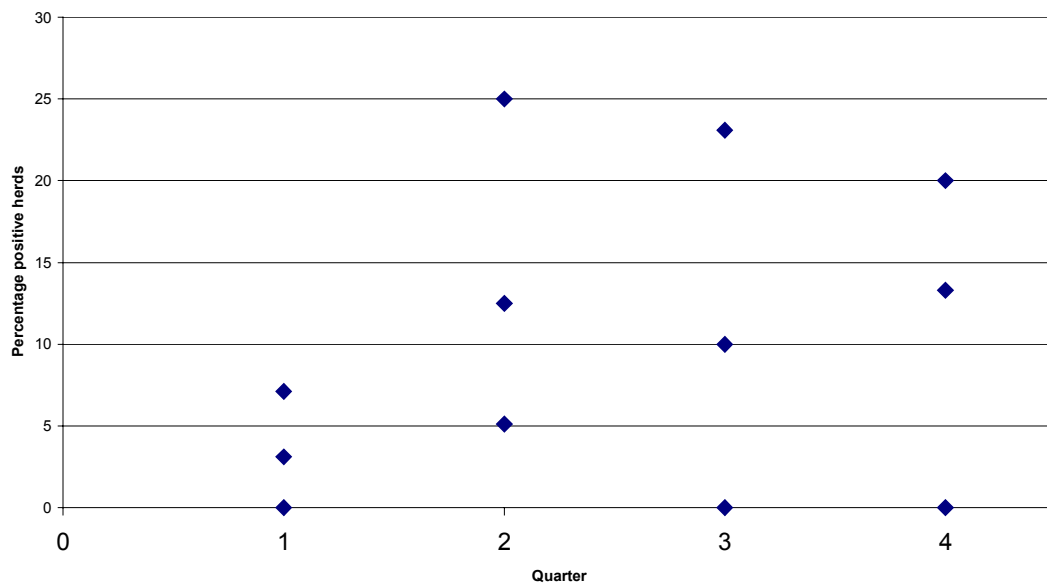


Fig. A2-5. STEC O157 surveillance data in veal calves. Relationship with quarter of the year, aggregated over the years.

2.1.F7

De Bodt 2000

Summary

Serotype

E. coli O157, base type for study is STEC O157, but testing for VT1/VT2 is not yet done.

Prevalence

Dairy cattle: 0 - 30 %

Calves: 0 %

Virulence-associated genes

testing for VT1/VT2/eae, but not yet done.

Detection method

Enrichment in mTSB+a followed by IMS and isolation on CT-SMAC.

Sampling material

Faeces from dairy cattle and calves.

Sampling strategy

All cattle present was sampled.

Sampling location

Dairy farm.

Period of sampling

2 July 1999 - present.

Herd size

56 - 119 dairy cattle and 5 – 19 calves, see ‘details-time dynamics’.

Geographic distribution

The farm is located in Driebergen-Rijsenburg.

Time dynamics

Sampling about once per month; cattle was monitored individually. See ‘details-time dynamics’.

Details

Time dynamics

Table A2-20 Prevalence of *E. coli* O157 at animal level on a farm, related to time.

Sampling date	Type	Total no.	No. positive	Percentage (%)
2 Jul 1999	Dairy cattle	68	20	30.3
2 Jul 1999	Calves	0	-	-
30 Jul 1999	Dairy cattle	59	12	20.3
30 Jul 1999	Calves	5	0	0.0
30 Aug 1999	Dairy cattle	56	7	12.5
30 Aug 1999	Calves	7	0	0.0
4 Oct 1999	Dairy cattle	61	1	1.6
4 Oct 1999	Calves	6	0	0.0
12 Nov 1999	Dairy cattle	68	0	0.0
12 Nov 1999	Calves	12	0	0.0
5 Jan 2000	Dairy cattle	118	0	0.0
5 Jan 2000	Calves	15	0	0.0
8 Feb 2000	Dairy cattle	119	0	0.0
8 Feb 2000	Calves	18	0	0.0
15 Mar 2000	Dairy cattle	116	0	0.0
15 Mar 2000	Calves	19	0	0.0

2.2 Prevalence at Slaughterhouse

2.2.S1

De Boer et al. 1994

Summary

Serotype

STEC O157, base type for article was *E. coli* O157:H7

Prevalence

No positives in 550 samples.

Virulence-associated genes

Not tested because no *E. coli* O157 were found.

Detection method

enrichment in TSB+a; isolation on SMAC.

This detection method is now outdated, but at that time the best and most used method (pers.comm. Heuvelink).

Sampling material

Faeces from the rectum of adult cattle (veal bulls and dairy cows; it is not known how many of each, pers.comm. Heuvelink) taken directly after slaughter.

Sampling location

Slaughterhouses.

Period of sampling

May-June 1993.

Geographic distribution

From throughout the Netherlands.

2.2.S2

Heuvelink et al. 1996

Summary

There is overlap with Heuvelink et al. 1998a, Heuvelink 2000g.

Serotype

STEC O157, base for article was also STEC O157.

Prevalence

Table A2-21 Prevalence of STEC O157 at animal level

Type	Period	No. positive samples	Total no. of samples	Percentage (%)
Veal calves	1994	3	365	0.9
Veal calves	1995	1	183	0.5
Adult cattle	1995	30	270	11.1

The positive veal calves all originated from different farms.

Virulence-associated genes

PCR for VT1, VT2 and eae.

Detection method

1994: enrichment in mTSB+a, isolation on SMAC and TC-SMAC.

1995: three methods were applied simultaneously:

enrichment in mEC+n for 18 to 20 h followed by isolation on SMAC, and

enrichment in mEC+n for 18 to 20 h followed by isolation on CT-SMAC, and enrichment in mEC+n for 6 to 8 h followed by IMS and isolation on CT-SMAC.

Sampling material

Faeces of veal calves and adult cattle, by a cut in the rectum directly after slaughter. Adult cattle originated from several dairy farms, veal calves arrived as a whole group originating from a farm. Adult cattle will mainly comprise of dairy cattle and further of bulls and veal cattle (pers.comm. Heuvelink). The names of the farms, group size and box or group housing was noted.

Sampling strategy

For veal calves, sample size was $\sqrt{(\text{herd size})}$.

Sampling location

Slaughter houses.

Period of sampling

29 August – 14 September 1994.

24 July – 30 October 1995.

Herd size

Herd size is noted but not given in the article.

Geographic distribution

1994: Sampling in one slaughterhouse.

1995: Sampling in slaughterhouses in different regions of The Netherlands (see 'details').

Details

Geographic distribution

Table A2-22 Prevalence of STEC O157 at animal level, related to geographical area.

Year	Type	Area	Sample no.	No. positive samples
1994	Veal calves	East	365	
1995	Veal calves	East	93	
1995	Veal calves	West	60	
1995	Veal calves	South	30	
1995	Adult cattle	East	90	12
1995	Adult cattle	West	30	2
1995	Adult cattle	North	90	9
1995	Adult cattle	South	60	7

2.2.S3

Heuvelink et al. 1998a

Heuvelink 2000g

Summary

There is overlap with Heuvelink et al. 1996

Serotype

STEC O157, base type for article was E. coli O157.

Prevalence

Table A2-23 Prevalence of STEC O157 at animal level.

type	No. of positive animals	No. of sampled animals	Percentage
Veal calves	2	397	0.5
Adult cattle	55	540	10.2

The majority of adult cattle comprised of dairy cows.

Virulence-associated genes

Of the *E. coli* O157-positive adult cattle, two were negative for VT genes but eae-positive (both from October 1996, from different sampling events, pers.comm. Heuvelink), the rest was positive for VT1 and/or VT2 and eae.

Detection method

SMAC: enrichment in mEC+n for 18 to 20 h followed by isolation on SMAC.

CT-SMAC: enrichment in mEC+n for 18 to 20 h followed by isolation on CT-SMAC.

IMS: enrichment in mEC+n for 6 to 8 h followed by IMS and isolation on CT-SMAC.

Sampling material

faeces (rectal contents).

Sampling strategy

10 % of the total number of veal calves from a herd was sampled, with a maximum of 10 calves. The 397 veal calves originate from 45 herds.

Sampling location

5 adult cattle and four veal calves slaughterhouses.

Period of sampling

24 July - 30 October 1995.

12 July – 22 October 1996.

Geographic distribution

- Slaughterhouses from different regions of the country, location not related to origin of animals.
- No marked geographic variation in the prevalence of *E. coli* O157 for adult cattle (Fig.1 of this reference).
- The two *E. coli* O157-positive VT-negative *eae*-positive adult cattle were sampled by employees from department East, which however does not imply that the cattle originated from this region (pers.comm. Heuvelink).
- The origin of veal calves was predominantly the central part of the country, where calf-fattening herds are most concentrated.

Details

Prevalence

Table A2-24 Prevalence of *E. coli* O157 at animal level, related to detection method.

Year	type	No. positive animals	No. of sampled animals	Percentage	SMAC	CT-SMAC	IMS
1995	Adult cattle	30	270	11.1	0	4	30
1995	Veal calves	1	183	0.5	0	1	0
1996	Adult cattle	27*	270	10.0	not done	4	27
1996	Veal calves	1	214	0.5	not done	0	1

*: Two adult cattle from October 1996 from different sampling events were negative for VT genes and *eae*-positive (pers.comm. Heuvelink).

Interpretation

The use of IMS resulted in a sevenfold increase in the rate of isolation of STEC O157 from adult cattle compared with the rate after plating onto CT-SMAC following selective enrichment.

The rate of isolation might be influenced by:

- 1) setting: dairy herd versus slaughterhouse
- 2) geographical variation
- 3) seasonal variation

The difference in rate of isolation between veal calves and dairy cattle might be related to:

- 1) difference in diet
- 2) orally administered antibiotics
- 3) handling: veal calves directly from fattening herd to slaughterhouse, adult cattle can pass several stations (risk of contact with infected animals)

2.2.S4

Reinders et al. 1997a

Summary

This is the same research as presented in Reinders et al. 1997b.

Serotype

STEC O157, base type for article was also STEC O157.

Prevalence

0 of 273 calves were positive

Virulence-associated genes

PCR for eae, VT1 and VT2.

Detection method

Enrichment in mTSB+a, IMS, isolation on CT-SMAC.

Sampling material

Faeces (intestinal content) from veal calves.

Sampling strategy

Usually 6 to 10 samples per herd, but sometimes 20, 30 or 40. Sampling was not random, but determined by housing and feeding characteristics.

Sampling location

One slaughterhouse in the Netherlands.

Period of sampling

July to November 1996.

2.2.S5

Reinders et al. 1997b

Summary

This is the same research as presented in Reinders et al. 1997a.

Serotype

STEC O157, basis type for article was also STEC O157.

Prevalence

0 of 273 samples were positive for VTEC O157.

Virulence-associated genes

PCR for VT1, VT2 and eae.

Detection method

Enrichment in mTSB+a; IMS; CT-SMAC.

Sampling material

Faeces of veal calves.

Sampling location

Slaughterhouse.

Period of sampling

July to November 1996.

2.2.S6

Reinders and Bijker 1999

Summary

The part of this study on dairy cattle and on research in positive suppliers is presented under the section 2.1 Farm.

Serotype

STEC O157, base for report was also STEC O157.

Prevalence

12 of 329 samples positive. 7 of 53 suppliers positive. Three from these suppliers were visited and all companies were positive.

Virulence-associated genes

Testing for VT1, VT2, eae with PCR and for EHEC-plasmide.

Detection method

Enrichment in mTSBa; IMS; isolation on CT-SMAC.

Sampling material

Intestine samples from “IKB” veal bulls after slaughter.

Sampling location

Two slaughterhouses in the Netherlands.

Period of sampling

June 1997 to September 1998.

Herd size

Some herd sizes from positive suppliers (see details).

Geographic distribution

Location of slaughterhouses and some positive suppliers.

Time dynamics

Limited information (see details).

Details*Prevalence*

Table A2-25 Prevalence of STEC O157 at animal level for veal bulls in slaughterhouse no. 1.

Period	No. of samples	No. positive samples
June 1997	44	1

For data from the positive supplier see 2.1.F2 Reinders and Bijker 1999 ‘Details-suppliers of positive veal bulls’.

Table A2-26 Prevalence of STEC O157 at animal level for veal bulls in slaughterhouse no. 2.

Period	No. of samples	No. positive samples	No. of suppliers	No. of positive suppliers
Nov 1997	40	6	6	2
July-Aug 1998	117	5	22	5
Sep 1998	128	0	25	0
Total	285	11	53	7

For data from the 2 positive suppliers of November 1997, see 2.1.F2 Reinders and Bijker 1999 'Details-suppliers of positive veal bulls'.

2.2.S7

Reinders 2000

Summary

Serotype

STEC O157, base type for study was also STEC O157.

Prevalence

No E. coli O157 and therewith no STEC O157 found, this implies that (see 'Sampling strategy' and 'Period of sampling') 0 of ca. 840 veal calves were positive and that 0 of ca. 28-56 herds were positive.

Virulence-associated genes

Not tested because no E. coli O157 were found.

Detection method

Enrichment in mTSB+a, IMS, CT-SMAC.

Sampling material

Intestine content of veal calves, samples taken after slaughter.

Sampling strategy

30 samples are taken per visit, from one or two herds.

Sampling location

One specific slaughterhouse with more than 100.000 slaughterings per year.

Period of sampling

End 1997 up to now and running, samples taken monthly.

Geographic distribution

Origin of the majority of the samples is traceable if necessary.

2.3 Prevalence at Retail

2.3.R1

De Boer et al. 1992

Summary

Serotype

E. coli O157 with verotoxicity, base type for article was E. coli O157.

Prevalence

E. coli O157: 3 of 69 samples positive. Of these 3, none were positive for verotoxicity.

Virulence-associated genes

Test for verocytotoxicity.

Detection method

Enrichment in mEC+n, 3 M Petrifilm Test Kit-HEC.

Sampling material

Raw beef.

Sampling location

Retail trade.

2.3.R2

Van Heerwaarden and De Boer 1993

Summary

Serotype

STEC O157, base for article was E. coli O157:H7.

Prevalence

0 of 264 samples positive.

Detection method

Enrichment in mTSB+A, isolation on SMAC+MUG and EMB, confirmation with O157-latex agglutination and API 20 E.

Sampling material

raw beef meat.

Sampling location

Retail trade.

Period of sampling

October 1992 to October 1993.

2.3.R3

Heuvelink et al. 1994

Summary*Serotype*

SLTEC, base type for article was SLTEC.

Prevalence

1 positive of 17 samples.

Virulence-associated genes

PCR for SLT1 and SLTII.

Detection method

Enrichment in mTSB+a; enrichment in BHI.

Sampling material

Tartare.

2.3.R4

Heuvelink et al. 1996

Heuvelink 2000e

Summary

This is the same research as 2.3.R5 De Boer et al. 1996.

Serotype

STEC O157, base type for article was STEC O157, E. coli O157 and VTEC.

Prevalence

survey 1: 0 of 1000 samples of raw minced beef were positive for STEC O157.

survey 2: 6 of 201 samples of raw minced beef were positive for E. coli O157, but they were all VT-negative, so there was no STEC O157 present.

survey 3: 92 samples of raw minced beef of survey 1 were tested for VTEC. 13 were positive.

In conclusion, we have the information:

0 of 1000 raw minced beef samples positive for STEC O157

0 of 201 raw minced beef samples positive for STEC O157.

Virulence-associated genes

Test for VT1, VT2 and Vero cell assay.

Detection method

- 1) enrichment in mTSB+a followed by isolation on SMAC. This detects STEC O157.
- 2) enrichment in mEC+n followed by the 3M Petrifilm Test Kit-HEC. This detects E. coli O157.
- 3) enrichment in mTSB+a followed by PCR for VT1 and VT2. This detects VTEC.

These measurements have a relatively low sensitivity as the IMS method is not used. The method used was at that time the most used and best method. The PCR method is a sensitive method.

Sampling material

raw minced beef (partly tartare)

Sampling strategy

3) is a random selection of 1). 2) is a separate set of samples.

Sampling location

Retail outlets (possibly including restaurants, pers.comm. Heuvelink).

Period of sampling

October 1992 until September 1995.

2.3.R5

De Boer et al. 1996

Summary

This is the same research as 2.3.R4 Heuvelink et al. 1996 Heuvelink 2000e.

Serotype

STEC O157, basis type for article was also STEC O157.

Prevalence

0 of 1000 samples raw minced beef were positive.

Virulence-associated genes

With PCR testing for eae, VT1 and VT2; also veroceltest for verotoxines.

Detection method

Enrichment in TSB+a; isolation on SMAC.

Sampling material

Raw minced beef.

Period of sampling

1993-1995.

2.3.R6

De Boer et al. 1997

Summary

This is the same research as 2.3.R7 Heuvelink et al. 1999a Heuvelink 2000f and 2.3.R8 Heuvelink 2000a.

Serotype

STEC O157, base type for article was also STEC O157.

Prevalence

Table A2-27 Prevalence of STEC O157 at product level.

Meat type	No. of samples investigated	No. of samples positive	Percentage
Tartare	39	1	2.6

Virulence-associated genes

Testing for virulence factors was done.

Detection method

Enrichment in mEC+n for 6 to 8 h followed by IMS and isolation on CT-SMAC.

Sampling material

Tartare.

Sampling location

Retail trade.

Period of sampling

1996.

Geographic distribution

Inspection area of the Inspectorate for Health Protection and Veterinary Public Health location Zutphen.

2.3.R7

Heuvelink et al. 1999a

Heuvelink 2000f

Summary

This is the same research as 2.3.R6 De Boer et al. 1997 and 2.3.R8 Heuvelink 2000a.

Serotype

STEC O157, base type for article was also STEC O157.

Prevalence

Table A2-28 Prevalence of STEC O157 at product level.

Product	Year	No. positive samples	Total no. of samples	Percentage (%)
Raw minced beef	1996	4	264	1.5
Raw minced beef	1997	2	307	0.7

Raw minced beef is partially tartare, see De Boer et al. 1997 Heuvelink 2000a.

Virulence-associated genes

All positive for VT2 and eae.

Detection method

Enrichment in mEC+n for 6 to 8 h followed by IMS and isolation on CT-SMAC.

Sampling material

Raw minced beef.

Sampling location

Supermarkets and butcher's shops (raw minced beef).

Period of sampling

13 March 1996 to 8 December 1997 (raw minced beef).

Time dynamics

Revisiting Butcher's shop I (first visit gave STEC O157-positive samples) 3 and 11 days later gave 44 STEC O157-negative results.

Revisiting Butcher's shop VI (visit on 8 December 1997 gave STEC O157-positive samples) six times resulted in two visits with positive samples followed by four visits with negative samples (see 'Details - Time dynamics').

Details*Time dynamics*

Table A2-29 Prevalence of STEC O157 at product level in Butcher's shop VI related to time.
?: total number of samples not given.

Date	Positive samples
23 May 1997	0/2
8 December 1997	2/?
15 December 1997	5/5
29 December 1997	2/5
9 January 1998	0/13
23 January 1998	0/6
27 February 1998	0/5
23 March 1998	0/5

Survival

- The STEC O157 test strain was able to survive in tartare stored at –20, 0, 5, or 7 °C for 3 days.
- STEC O157 counts in tartare hardly changed for 5 days at 7 and 15 °C.

Transmission

1/28 cows of the farm that delivered the cow producing positive beef samples in butcher's shop VI was STEC O157-positive. But strain type did not match.

2.3.R8

Heuvelink 2000a

Summary

This is the same research as 2.3.R6 De Boer et al. 1997 and 2.3.R7 Heuvelink et al. 1999a Heuvelink 2000f.

Serotype

STEC O157, comparison with De Boer et al. 1997 and Heuvelink et al. 1999a Heuvelink 2000f shows that Heuvelink 2000a also is about STEC O157.

Prevalence

Table A2-30 Prevalence of STEC O157 in tartare at product level

Year	No. positive	Total No.
1996	1	39
1997	0	43

Sampling material

Tartare

Period of sampling

1996 en 1997.

Geographic distribution

Numbers from the Inspectorate for Health Protection and Veterinary Public Health, department Zutphen.

2.4 Prevalence Reviews

Three reviews, thus not containing original data were found. These are shown below, citing the references that were used. In a number of cases the data given in the reviews do not match with the original data in the references used.

2.4.1

Anonymus 1998

Data used

2.3.R6 De Boer et al. 1997

2.3.R8 Heuvelink 2000a

2.3.R7 Heuvelink 2000f

2.1.F3 Heuvelink et al. 1999b, but data do not match.

2.1.F1 Heuvelink 2000d

2.2.S3 Heuvelink 2000g

2.4.2

Herbes et al. 1999

Data used

2.2.S1 De Boer et al. 1994

2.3.R4 Heuvelink et al. 1996

2.2.S3 Heuvelink 2000g

2.1.F1 Heuvelink 2000d, but very many differences in numbers

2.3.R4 Heuvelink 2000e

2.3.R5 De Boer et al. 1996

2.3.R6 De Boer et al. 1997

2.3.R7 Heuvelink 2000f, data 1997 do not match

2.4.3

Visser et al. 1999

Data used

p. 9:

data 1997: 2.1.F3 Heuvelink et al. 1999b, data do not match.

data 1998: 2.1.F4 Van de Giessen 1998

Table 11.1:

2.2.S1 De Boer et al. 1994

2.2.S3 Heuvelink 2000g

2.3.R4 Heuvelink et al. 1996

Table 11.2:

2.1.F1 Heuvelink 2000d

2.1.F4 Van de Giessen 1998

Table 11.3:

2.3.R4 Heuvelink 2000e

2.3.R6 De Boer et al. 1997

2.3.R7 Heuvelink 2000f, data 1997 do not match

2.3.R5 De Boer et al. 1996

Table 11:

2.1.F4 Van de Giessen 1998

2.5 Concentration in faeces

A recent review on concentration of *E. coli* O157 in faeces is Wallace 1999. Most literature data on this subject deal with experimentally infected cattle. These references are given below, with a short summary of numerical values.

Brown et al. 1997

Cattle. Max 4×10^6 CFU/g to detectable only by enrichment from 20 days onwards.

Cray and Moon 1995

First 10 days: calves $10^4 - 10^9$ CFU/g, adults $10^3 - 10^7$ CFU/g. Thereafter: calves up to 10^4 , adults up to 10^2 CFU/g for 7 – 20 weeks.

Harmon et al. 1999

Calves. Max ca. 10^8 CFU/g to fluctuating between ca. 3×10^3 and undetectable from 14 days onwards.

Zhao et al. 1998

Calves. From 10^5 to 10^8 on day 2 postinoculation to 10^3 to enrichment detection from day 10 to day 32.

Two references deal with naturally infected cattle, which is more useful:

Shere et al. 1998

Cattle. 200 to 87000 CFU/g *E. coli* O157:H7.

Zhao et al. 1995

Cattle. <100 to 10^5 CFU/g.

This reference is used e.g. by Cassin et al. 1998, Coleman et al. 1998, Daube and Wauters 1998.

2.6 Concentration on carcasses

Daube and Wauters 1998 give preliminary results on EHEC O157. This are data from the Institute of Veterinary Inspection in the Ministry of Public Health. Considering the proved cases, assuming that one carcass is contaminated in each pool of 5, a contamination of 1 cfu/cm² and 200 cfu/cm² was registered for 2 of the 3 positive cases.

2.7 Concentration in ground beef

References on *E. coli* O157 counts in ground beef are given below, with a short summary of the results.

Armstrong et al. 1996

(p. 34). Large outbreak in 1993, hamburger patties had ≤ 700 organisms each before cooking. Griffin 1995 is given as a reference but this does not give this information.

(p. 37). Outbreak ground beef in the freezer: 500-1000 CFU/g. Other side of the cow: 100 CFU/g. Finelli et al. 1995 is given as a reference but this does not give this information.

Padhye and Doyle 1991

A survey of ground beef from Madison area grocery stores gave 3 *E. coli* O157:H7-positive samples of a total of 107 samples. MPN gave 0.4 to 1.5 cells per g for these samples.

Coleman et al. 1998

(page A-3). Data from MPN enumeration from the 1993 outbreak in the western states: of six samples analysed, most probable numbers per gram for 25-gram samples of raw ground beef, unadjusted for recovery from frozen product, were 0.3 – 15 MPN/g.

2.8 Transmission of micro-organisms in Slaughterhouse

A limited number of references on transmission of micro-organisms in slaughter houses was found, but it must be noted that references on this subject were not specifically searched for. The references with a short summary are given below.

Bell 1997

Sites without faeces/clean hide contact: APC $< \log 2$ CFU/cm²; *E. coli* $< \log 1$ CFU/cm²;

Sites contacted by clean hide: APC $> \log 3$ CFU/cm²; *E. coli* $< \log 2$ CFU/cm²;

Sites with faeces/dirty hide: APC $> \log 4$ CFU/cm²; *E. coli* $> \log 2$ CFU/cm².

Chapman et al. 1993

Carcasses from 7 (30%) of 23 rectal swab-positive cattle and 2 (8%) of 25 rectal swab-negative cattle were positive.

Donkersgoed et al. 1997

No consistent association between tag scores (mud, bedding, manure) and bacterial contamination of carcasses.

Gill et al. 1996

Counts of aerobic flora, coliforms and *E. coli* after skinning, carcass splitting, trimming and washing at neck, brisket and rump. In total 3 sites x 4 points x 24 samples per micro-organism group.

Gill CO et al. 1998

Counts of aerobic flora, coliforms and *E. coli* related to skinning the beef carcass hindquarters for three slaughtering plants. Differences between plants, operations and sites.

Appendix 3 *Mixing and partitioning: Modelling non-random distribution in food handling processes*

Mixing

In the ‘mixing’ basic process small units are gathered to form a large unit. If the numbers of cells (particles) on the small units are known, the total number in the large unit is the sum of the numbers on the small units ($N = \sum_i N_i$). Often, however, the numbers on the small units will not be known exactly. When the small units added are samples from a large population of small units and the distribution of the N_i 's in that large population is known, the N_i 's added are samples from a distribution and the sum is the sum of random samples from that distribution (see Nauta 2001b).

A complicating factor may be that the small units have unequal sizes. As in steps 1 and 5 in the present risk assessment model (section 3.3), the (variability distribution of) the size of large unit(s) may be known, and (the variability distribution of) the number of small units contributing may be known, but not the (relative) sizes of the small units. If, for example, a tartare holds beef from k different carcasses, (or if a carcass holds faeces from k different animals,) we need a ‘random distribution’ that describes how much of the tartare originates from each carcass. The easiest thing is to assume that all carcasses contribute equally, but this is probably not the case: some contribute more than others. Therefore we need an algorithm to describe the relative sizes of the small units in which the number of small units is predefined, and the sum of relative sizes is one, and in which (preferably) the size of each small unit has the same probability distribution.

This problem is rephrased by looking back, as follows: A (continuous) quantity Q , has to be distributed at random in k parts. We require that the size of each part q_i ($i=1..k$) should be a sample from the same distribution and that the order in which the samples are taken should have no influence on their size. That means, for example, that the largest part may be drawn at any moment, with equal probability. We now require a probability distribution /algorithm that allows us to do this.

Here we propose to use the following algorithm:

part 1 is a sample from Beta ($b_f, b_f(k-1)$): x_1 ; The size of part 1 is $q_1 = x_1 Q$
 part 2 is a sample from Beta ($b_f, b_f(k-2)$): x_2 ; The size of part 2 is $q_2 = x_2 (1-x_1)Q$
 ...
 part y Beta($b_f, b_f(k-y)$): x_y ; The size of part y is $q_y = x_y \prod_i (1-x_i) Q$
 ...
 part k has size $q_k = \prod_{k-1} (1-x_i) Q$

with b_f (the “beta factor”) a positive number

So in short, for $i = 1..k$

$$\begin{aligned} x_i &\sim \text{Beta}(b_f, b_f(k-i)) \\ q_i &= Q x_i \prod_{j=1}^{i-1} (1-x_j) \end{aligned} \quad (\text{A3.1})$$

with $\text{Beta}(b_f, 0) = 1$.

In this algorithm the parameter b_f determines the variability between the q_i 's, the sizes of the parts that are mixed. With an increase of b_f the variability decreases. If b_f is infinitely large, all $q_i = Q/k$, while if $b_f = 0$, one random $q_i = Q$, all other $q_i = 0$.

It is easy to see that the mean of each part is Q/k , as it should be:

The mean of $\text{Beta}(b_f, b_f x) = 1/(x+1)$. So part 1 has mean size $E(q_1) = Q/k$, part two has size $q_2 = 1/(k-1) \times (k-1)/k \times Q = Q/k$, etc...

It is not so easy to see that the same is true for the variance. However, numerical analysis of the algorithm given has shown that this is the case in all trials we performed (*data not shown*). The relative size (q_i/Q) has the same probability distribution for each part $i = 1..k$ for given values of b_f and k .

Next, we can proof analytically that the algorithm yields equal probability distributions for the q_i for the simple case that if $b_f = 1$, and for $i=1$ and $i=2$ only, as follows:

As the probability density function the $\text{Beta}(1, k-1)$ distribution equals $f(x) = (k-1)(1-x)^{k-2}$,

$$P(x_1 < \rho) = \int_0^\rho (k-1)(1-x)^{k-2} dx = 1-(1-\rho)^{k-1} \quad (\text{A3.2})$$

This $P(x_1 < \rho)$ should equal $P(x_2(1-x_1) < \rho)$ for any ρ . Note that $P(x_2(1-x_1) < \rho)$ depends on $P(x_1=p)$ (for all $0 < p < 1$). For $x_1 = p$, with $P(x_2(1-x_1) < \rho)$ we request $P(x_2 < \rho/(1-p))$. To calculate $P(x_2(1-x_1) < \rho)$, we need to integrate this probability over all values of p multiplied with the probability that $x_1 = p$.

We consider two cases:

1) If $p > 1-\rho$, $P(x_2 < \rho/(1-p)) = 1$ (because $x_2 \leq 1$)

2) If $p \leq 1-\rho$, $P(x_2 < \rho/(1-p)) = \int_0^{\rho/(1-p)} (k-2)(1-x)^{k-3} dx$.

(As x_2 is a sample of $\text{Beta}(1, k-2)$ with probability density function $f(x) = (k-2)(1-x)^{k-3}$.)

So

$$P(x_2(1-x_1) < \rho) =$$

$$\int_0^1 P(x_1 = p) P(x_2 < \frac{\rho}{1-p}) dp =$$

$$\int_0^1 (k-1)(1-p)^{k-2} P(x_2 < \frac{\rho}{1-p}) dp =$$

$$\int_0^{1-\rho} (k-1)(1-p)^{k-2} \left(\int_0^{\rho/(1-p)} (k-2)(1-x)^{k-3} dx \right) dp + \int_{1-\rho}^1 (k-1)(1-p)^{k-2} dp =$$

$$\int_0^{1-\rho} (k-1)(1-p)^{k-2} \left(1 - \left(\frac{1-p-\rho}{1-p} \right)^{k-2} \right) dp + \rho^{k-1} =$$

$$1 - (1-\rho)^{k-1}.$$

Q.E.D.

Using this methodology (A3.1), the relative contribution of each of the k small units, q_i/Q , can be illustrated with a pie chart. By ranking the values of q_i/Q and calculating the means of the largest, the second largest, etc., one gets an impression of the mean relative distribution sizes. For several combinations of k and b_f , the result is illustrated in Figure A3-1.

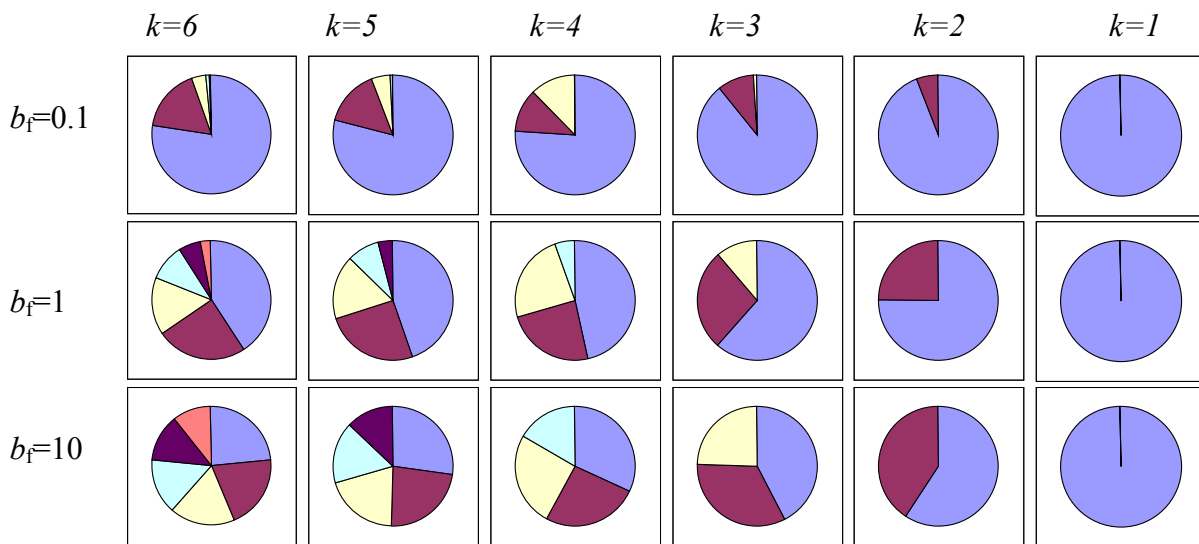


Figure A3-1 The mean distribution of relative contributions q_i/Q to a mixed unit with size Q , for different values of beta factor b_f and the number of contributing units k . With smaller beta factor, the variability in sizes increases.

Partitioning

Likewise, in a partitioning process, with random sampling, for a series of $i=1..x$ equal sized smaller units, there is a problem of dependence between the number of cells found the samples. These numbers can be given as follows (Nauta 2001b):

$$N_i' \sim \text{Binomial} \left[N - \sum_{j=1}^{j=i} N_j', 1/(x-i+1) \right] \tag{A3.3}$$

as for a series of i , as in the following list (with $p = i/x$):

$$\begin{aligned}
i=1: & N_1' = \text{Bin}(N, p), \\
i=2: & N_2' = \text{Bin}(N - N_1', p/(1-p)), \\
i=3: & N_3' = \text{Bin}(N - N_1' - N_2', p/(1-2p)) \\
& \dots \\
i=j: & N_j' = \text{Bin}(N - \sum_{i=1}^{j-1} N_i', p/(1-(j-1)p)) \\
& \dots \\
i=x: & N_x' = N - \sum_{i=1}^{x-1} N_i'
\end{aligned}$$

This series assumes an equal p for each smaller unit. However, if clustering occurs or if the sizes of the small units are variable, this p will not be equal for each unit. This implies that p has a probability distribution. Assume that for x small units originating from one large unit, p has a Beta distribution $\text{Beta}(b, b(x-1))$. Then, the numbers of cells in the small units are samples from a Betabinomial distribution

$$N_i' \sim \text{Binomial}[N - \sum_{j=1}^{i-1} N_j', \text{Beta}(b, b(x-i))], \quad (\text{A3.4})$$

with a parameter b describing the amount of clustering, such that there is full clustering when b approximates zero and no clustering (that is random distribution with a constant p) when b approximates infinity.

This implies a series of $i = 1..x$, as in the following list (with $p = i/x$):

$$\begin{aligned}
i=1: & N_1' = \text{Bin}(N, \text{Beta}(b, b(x-1))), \\
i=2: & N_2' = \text{Bin}(N - N_1', \text{Beta}(b, b(x-2))), \\
i=3: & N_3' = \text{Bin}(N - N_1' - N_2', \text{Beta}(b, b(x-3))) \\
& \dots \\
i=j: & N_j' = \text{Bin}(N - \sum_{i=1}^{j-1} N_i', \text{Beta}(b, b(x-j))) \\
& \dots \\
i=x: & N_x' = N - \sum_{i=1}^{x-1} N_i'
\end{aligned}$$

Clearly, this resembles the algorithm (A3.1) as given above for mixing.

Using this methodology the units 1 to x initially have equal probability distributions for N_i , ($N_i' = \text{Bin}(N, \text{Beta}(b, b(x-1)))$ with mean N/x and variance $N(x-1)(bx+N)/(x^2(bx+1))$). The parameter b is easily interpreted as a clustering parameter.

When considering the change in prevalence as a consequence of partitioning with clustering, it is of interest to calculate the probability that a small unit from a large unit containing N cells is empty, that is $P(N_i = 0)$.

Considering that the probability density function of the Beta-Binomial distribution (n, α, β) is (Vose 2000)

$$f(x) = \binom{n}{x} \frac{(\alpha + x - 1)!(n + \beta - x - 1)!(\alpha + \beta - 1)!}{(\alpha + \beta + n - 1)!(\alpha - 1)!(\beta - 1)!}.$$

and knowing that for non-integer values $n! = \Gamma(n+1)$, the probability that $N_i = 0$ can be calculated as

$$P(N_i = 0) = \frac{\Gamma(bx)\Gamma(N + b(x-1))}{\Gamma(b(x-1))\Gamma(bx + N)} = \frac{\text{Beta}(bx, N)}{\text{Beta}(b(x-1), N)}$$

which in the special case that $b=1$ simplifies to $P(N_i = 0) = (x-1)/(N+x-1)$.

(Note that Beta stands for the Beta function here, not for the Beta distribution. Therefore $P(N_i = 0)$ is a number, not a distribution.) The effect of applying this approach (A3.4) and not the Binomial distribution (A3.3) depends on the effect that it has on the variance of the number of cells per units.

The variance in case of the betabinomial model is $\text{var}_{bb}(N_i) = \frac{N(x-1)(bx+N)}{x^2(bx+1)}$

whereas the variance in case of the binomial model with $p = 1/x$ is $\text{var}_{bi}(N_i) = \frac{N(x-1)}{x^2}$.

Hence, $\frac{\text{var}_{bb}(N_i)}{\text{var}_{bi}(N_i)} = \frac{bx+N}{bx+1} = 1 + \frac{N-1}{bx+1}$, which indicates that the larger N and the smaller bx , the larger the impact of clustering.