

TECHNICAL REPORT submitted to EFSA
A quantitative microbiological risk assessment of *Campylobacter* in the broiler meat chain¹

Prepared by Vose Consulting (US) LLC
1643 Spruce Street, Boulder, Colorado, 80302, USA

In collaboration with:

The Danish Agriculture and Food Council
The Faculty of Veterinary Medicine of the Technical University of Lisbon
The Faculty of Veterinary Medicine of the University of Thessaly

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1 Executive summary

This report describes the model produced by Vose Consulting to evaluate quantitatively the effect of interventions on the risk of campylobacteriosis from broiler meat in EU Member States (MS). The report provides details of the model structure and mathematics, assumptions, and how the user can assess various interventions. It also describes how data can be added to or excluded from the model, and how to interpret the results produced by the model. This report also illustrates the use of the model through a number of intervention scenarios for various countries.

The model has been developed by Vose Consulting in collaboration with the Danish Agriculture and Food Council, the Faculty of Veterinary Medicine of the Technical University of Lisbon, the Faculty of Veterinary Medicine of the University of Thessaly. This collaboration allowed a timely availability of necessary data and a constant critical review of the model development by experts from different regions in the EU.

This model has been designed to evaluate as efficiently and robustly as possible the interventions proposed by the Working Group of the EFSA BIOHAZ panel on *Campylobacter* in broiler meat - Risk assessment and control options (EFSA BIOHAZ WG). The model uses many of the same principles of previous food safety risk assessment models, but takes a different mathematical approach to achieve its results. This provides the ability to investigate the effect of different combinations of interventions extremely quickly.

A fairly unique feature of the model is that it is normalized to current observations throughout the farm-to-fork continuum. It also estimates the *change* in the human incidence rate of campylobacteriosis, rather than the actual incidence rates before and after variations in the interventions applied. The main advantage of this approach is that the model's outputs are less sensitive to any assumptions or statistical uncertainty in parameter estimates, leading to more robust quantitative results.

This report does not describe the results of any risk assessment study. The modeling results which are provided are purely illustrative. In order to demonstrate the use of the model, it was necessary for us to find references with data that could be representative of the effectiveness of the various interventions. Nonetheless, we have done our best to find references that we believe could be appropriate. The use of these references does not imply any endorsement of their validity by EFSA or the EFSA BIOHAZ WG. The model has the capacity to allow the user to add new references and remove the references that we have used. We are aware that any illustrative scenarios which have been evaluated in this report may not identify the most appropriate interventions or accurately reflect the status of the different MS.

2 Scope of the contract

In 2008 most MS carried out a harmonized baseline survey on the prevalence and antimicrobial resistance of *Campylobacter* spp. in broiler flocks and broiler carcasses (CEC, 2007). On carcasses, both qualitative and quantitative analyses were performed. The aim of the survey was to provide reference values, comparable between MS, in order to consider future performance objectives/targets along the broiler meat production chain.

The European Commission can consider:

- performance objectives/ targets for the reduction of the prevalence of *Campylobacter* at the level of primary production and, where appropriate, at other stages of the food chain (CEC, 2003);
- microbiological criteria for broiler meat where both food safety and process hygiene criteria may be considered (CEC, 2004, 2005).

Before deciding on risk management measures and setting performance objectives and/or targets, the Commission may carry out a cost-benefit analysis. The Commission has requested EFSA to help with the decision making process and the latter has contracted Vose Consulting to develop a quantitative model to assess the public health benefit resulting from potential control options. The model should serve as a tool for the Working Group of the EFSA BIOHAZ panel on *Campylobacter* in broiler meat - Risk assessment and control options (EFSA BIOHAZ WG), in order to formulate their opinion.

According to the technical annex of the contract, the model should be capable of doing the following:

- Carry out a quantitative microbiological risk assessment (QMRA) at the EU level and based on select existing models, regarding *Campylobacter* spp. in the broiler meat chain from primary production to consumer level.
- Provide the basis for defining potential performance objectives and/or targets at different stages of the food chain in order to obtain e.g. 50% and 90% reduction of the prevalence of human campylobacteriosis throughout the EU caused by broiler meat consumption or cross-contamination from broiler meat.
- If possible given available data, the performance objectives will include targets for reduction at pre-harvest and/or microbiological criteria for foodstuffs (qualitative or quantitative criteria for *Campylobacter* in general or for certain species or strains, e.g. those resistant to certain antibiotics).

At the first meeting with the EFSA BIOHAZ WG, the modeling approach was adjusted to the specific needs of the working group and following specific objectives were identified:

- The model will consider the broiler meat production pathway from the primary production until the end of the slaughter process, including interventions which are carried during carcass processing (e.g. freezing). The impact of interventions on *Campylobacter* contamination on the carcasses on the remaining broiler meat production pathway until consumption will not be considered by the model, as it is expected to be very limited.
- The model will be developed based on data available for 5 countries: Belgium, Denmark, Greece, Norway, and Portugal. In addition, a 'generic' country will be considered, comprising all best available data.
- The model will focus on essential input parameters by considering the Critical Control Points (CCP) from European HACCP systems.
- The model will consider a choice of intervention scenarios which will be elaborated by the EFSA BIOHAZ WG.

- The model should consider both farms where the birds are reared inside and farms where the birds have outdoor access.
- Mechanistic models were investigated but no data were available to quantify the parameters.

3 Project management

3.1 Project team

Given the short term of the contract and the high risk for not obtaining the necessary data in time, Vose Consulting subcontracted the Danish Agriculture & Food Council, the Faculty of Veterinary Medicine of the Technical University of Lisbon and the University of Thessaly to provide all available data for their country to the project and to review the model. The Project Team consisted of following experts:

Vose Consulting (US) LLC

- David Vose, Senior Partner
- Koen Mintiens, Senior Risk Analysis Consultant
- Michael Van Hauwermeiren, Risk Analysis Consultant
- Daan Raman, IT developer

Danish Agriculture and Food Council

- Lis Alban, Chief Scientist
- Marianne Sandberg, Researcher

Faculty of Veterinary Medicine of the Technical University of Lisbon

- Yolanda Vaz, Lecturer of Veterinary Public Health
- Maria João Fraqueza, Lecturer of Technology of Animal Products

Faculty of Veterinary Medicine of the University of Thessaly

- Leonidas Leontides, Associate Professor of Veterinary Epidemiology
- Polychronis Kostoulas, Lecturer in Veterinary Epidemiology.

Following experts represented EFSA and the EFSA BIOHAZ WG in the Project Team

- Arie Havelaar, Professor in Microbial Risk Assessment, IRAS, Utrecht University, NL
- Maarten Nauta, Senior researcher. DTU Food, DK
- Winy Messens, Scientific Attache, ILVO, BE
- Michaela Hempen, Scientific Officer, EFSA

At the start of the contract, the Project Team discussed the project organisation during a kick-off meeting at in Brussels (12-13 October 2009). Following issues were addressed:

- General outline of the project
- Project management and communication
- Model development & interventions
- Data collections
- Project Planning and to do's

Following that meeting, the progression of the project was discussed during weekly telephone conferences. During the second Project Team face-to-face meeting in Ghent (13-14 January 2010) following items were discussed.

- Discussion on the model development
- Discussion on the intervention list
- Data quality assessment

- Assumption assessment
- Project Planning and to do's

After the second face-to-face meeting, communication within the Project Team was maintained through two-weekly telephone conferences.

3.2 Project website

In addition to the Project Team meetings, a project website was developed to serve as an important communication tool. The website was developed using the CoMindWork online project management application (www.comindwork.com). The application includes all standard project management features and makes them accessible to all the members of the project. In addition, the application has an email notification system for reminding the Project Team members about tasks, events, deadlines etc.

For this project following features were provided:

- Description of the project goal
- Register of the Project Team members
- To do lists
- Milestones and deliverables
- Gantt chart describing the timing of the tasks
- Cases were created to manage tasks that involved different Project Team partners
- Wiki's were created to initiate specific discussions
- Files could be uploaded (e.g. references, tables, ..) for distribution

3.3 Project board

The progression of the project has been discussed with the EFSA BIOHAZ WG at 4 meeting. The topics that have been discussed during these meeting are stated below.

1st Meeting with the EFSA BIOHAZ WG, 20 November 2009, Parma, Italy

- Data collection by Project Team partners
- Modelling approach
- Interventions to be considered
- Quality assessment
- Tasks and planning

2nd Meeting with the EFSA BIOHAZ WG, 26 January 2010, Brussels, Belgium

- The logic behind the model
- The interventions
- Assumption assessment
- Data quality assessment
- Tasks and planning

3rd Meeting with the EFSA BIOHAZ WG, 2 March 2010, Copenhagen, Denmark

- Evaluation of the draft model
- Evaluation of the draft report

4th Meeting with the EFSA BIOHAZ WG, 20 April 2010, Telephone conference

- Update on model development
- Using the model for intervention assessment
- Using the model for target or criteria setting

The one day training session on model application was provided on 10 May 2010 at EFSA in Parma by David Vose and Michael Van Hauwermeiren.

4 Data collection

4.1 Data provided by EFSA

4.1.1 The EFSA baseline survey

Several data inputs in the model were obtained from EFSA from the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses that was carried out in the EU in 2008 (EFSA, 2010). The survey was conducted at the broiler batch level in slaughterhouses in 26 EU MS, plus Norway and Switzerland, whereas Greece did not carry out the survey. A detailed description of the design of the baseline survey, the sample design, the sample sizes and the bacteriological analyses can be found in the Commission Decision 2007/516/EC6 (CEC, 2007).

A first data input in the model is the (between) flock/batch prevalence obtained from detecting *Campylobacter* in a caecal content sample pooled from 10 individual birds per batch, randomly selected during the slaughter process. Isolation and confirmation of *Campylobacter* organisms in the caecal contents were undertaken as described in ISO 10272-1 (ISO, 2006a).

A second data input is the *Campylobacter* carcass concentration obtained from detecting and enumerating *Campylobacter* in a skin sample obtained from 1 carcass per batch, randomly selected after the chilling process. Isolation and confirmation of *Campylobacter* organisms on the broiler carcass samples were again undertaken as described in ISO 10272-1. The quantitative analysis of *Campylobacter* in the broiler carcass samples was carried out according to ISO/TS 10272-2 (ISO, 2006b).

A third input is the number of broilers slaughtered in the different countries during 2008. All countries provided their best estimates, which originated in some cases from 2007 data (Germany) or total weight of slaughtered broilers during 2008 (Bulgaria, Hungary). It should be noted that some of these figures may be overestimates as they also include cockerel, capons, poulardes (France) and cast (spent) hens and other poultry (excluding turkeys) weighing less than 2 kg respectively (United Kingdom).

4.1.2 Human incidence data

Data on campylobacteriosis in humans were obtained from the Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union. The report summarizes the numbers of confirmed human campylobacteriosis cases notified by the MS.

In a first stage data for 2007 were used but these were later updated to 2008 as the 2008 Community Summary Report became available. It must be noted that the report did not provide data for Greece and Portugal, but human campylobacteriosis incidence data for these countries were obtained elsewhere (see below).

4.2 Data from Belgium

4.2.1 *Campylobacter* prevalence or concentration data

Campylobacter prevalence and concentration data were obtained from the Belgian Federal Agency for the Safety of the Food Chain. The data result from the control programmes carried out by the Agency in all slaughterhouses and cutting, processing and distribution plants.

4.2.2 Intervention data

Information on the level of application of the different interventions that are applied in the model was obtained by contacting several Belgian experts in the broiler meat production chain. This information was included in the model and used for evaluating the future scenarios for Belgium.

4.2.3 Human incidence data

Human incidence data are summarised by the Belgian Federal Institute for Public Health. These data are provided to the ECDC and consequently summarized in the *Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union*. These summary data were used in the model and therefore no additional human incidence data were obtained for Belgium.

4.3 Data from Denmark

4.3.1 *Campylobacter* prevalence or concentration data

4.3.1.1 At farm level

Danish poultry farms delivering to the Lantmannen Danpo A/S and Rose Poultry A/S slaughterhouses sample their flocks using two socks per chicken house for the detection of *Campylobacter* using PCR. Between 2005 and 2009 these samplings were conducted on a voluntary base but from 2010 it is mandatory. The samples are used as a tool in the Danish Action programme against *Campylobacter* in chicken meat which aims at sorting flocks into positives and negatives (including all chickens below the age of 50 days). Positive flocks will be subjected to risk-reducing measures like freezing or heat treatment to the widest extent possible with the aim of reducing the number of contaminated carcasses on the market. In Denmark, the sock samples are required to be submitted to the laboratory at maximum of 8 days before slaughter. The data from this sampling procedure are owned by farmers delivering to Lantmannen Danpo A/S and Rose Poultry A/S.

4.3.1.2 At slaughterhouse level

For Danish poultry farms delivering to Lantmannen Danpo A/S and Rose Poultry A/S slaughterhouses cloaca swab samples are collected since 2007/2008 to estimate the *Campylobacter* prevalence in the chicken flocks using PCR. Rose Poultry A/S collects and pools 10 samples per flock, whereas Lantmannen Danpo A/S collects and pools 25 samples per flock. The samples are a part of the Danish Action programme against *Campylobacter* and owned by Danish Veterinary and Food Administration.

4.3.2 Intervention data

Some of the interventions that are used in the model are currently only applied in Denmark or Norway, e.g. sorting flock into positive and negative before slaughter. In the model, the effects of the interventions obtained from the Danish chicken farms were assumed to be possible to extrapolate for all countries.

Data on the level of application of the different interventions in Denmark were obtained from the veterinarians/health personnel working within Lantmannen Danpo A/S and Rose Poultry A/S.

4.3.3 Human incidence data

Human incidence data describing date of onset, age and sex of case were obtained from Statens Seruminstitut. The case definition included a positive culture. Underreporting is regarded to be a problem. The data are owned by the Danish Public Health Authorities, provided to the ECDC and consequently

summarized in the *Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union*.

4.4 Data from Greece

4.4.1 *Campylobacter* prevalence or concentration data

Data on *Campylobacter* prevalence in the primary production in Greece were obtained from a recent report from the occurrence of *Campylobacter* at slaughterhouse sampling points in Greece (Voidarou et al., 2007).

Information on *Campylobacter* prevalence at retail level was found in a recently published report on the *Campylobacter* spp. positive meat selling points in Northern Greece (Petridou and Zdragas, 2010).

4.4.2 Intervention data

Information on the level of application of the different interventions that are applied in the model were obtained by a questionnaire survey. The questionnaire was disseminated to 10 colleagues/experts supervising 95% of the Greek primary broiler production and slaughterhouses. They were specifically asked for each intervention on (a) the percentage of the farms/slaughterhouses under their supervision that were applying each intervention, (b) how effective and (c) how feasible the application of each intervention is (on a scale from 0 to 5, with 0 allocated to minimum and 5 to maximum effectiveness/feasibility).

Subsequently, the most likely (mode), minimum and maximum levels of application was calculated for all parameters/interventions.

4.4.3 Human incidence data

Collection of human incidence data was based on published work in peer reviewed journals, which reported on the incidence of human campylobacteriosis in different regions of Greece (Samonis et al., 1997; Kafetzis et al., 2001; Maltezou et al., 2001; Maraki et al., 2002).

Furthermore, the National Centre for Control and Prevention of Infectious Disease (N.C.I.D.C.P.) was contacted. This Centre provided the yearly incidence of confirmed *Campylobacter* spp. cases in humans. The N.C.I.D.C.P. experts stressed the well-known serious underreporting of campylobacteriosis. Hence, we also contacted the National Statistical Service of Greece (N.S.S.G.) to obtain demographic data, data on the yearly number of hospitalized patients and the patients identified with gastrointestinal infections whom were not fully investigated to identify the responsible microorganism. These data along with the data on the confirmed cases and the published estimates of the underestimation of human campylobacteriosis were used to quantify the actual number of human campylobacteriosis cases in Greece.

4.5 Data from Norway

4.5.1 *Campylobacter* prevalence or concentration data

The action plan regarding *Campylobacter* in Norwegian broilers was implemented in the spring of 2001. The objective is to reduce the human exposure to *Campylobacter* through Norwegian broiler meat products. The action plan is a joint effort involving several stakeholder groups from "stable-to-table" - the Norwegian Food Safety Authority, the National Veterinary Institute, the Norwegian Institute of Public Health, the Norwegian School of Veterinary Science, the Centre for Poultry Science, and the poultry

industry. The Norwegian Zoonosis Centre at the National Veterinary Institute coordinates the programme, and is responsible for the collection and analysis of data and dissemination of results.

As part of this Action Plan, all Norwegian broiler flocks has to be tested for *Campylobacter* spp. In the period 2001-2007, all flocks were sampled both at farm and again at slaughter. In 2008, the flocks were only sampled at farm.

Carcasses from flocks being positive at the sample taken at farm before slaughter have to be either heat treated or frozen for a minimum of three weeks before being marketed.

4.5.1.1 At farm level

For each flock, ten swabs from fresh fecal droppings are taken by the owner. The ten swabs are pooled and submitted to the laboratory where the samples are analyzed by real-time PCR (before 2005 analyzed by cultivation). These samples have to be taken maximum four days before slaughter (before 2005 maximum eight days before slaughter).

4.5.1.2 At slaughterhouse level

In the period 2001 – 2007, all flocks were sampled at slaughter (pooled sample consisting of caeca from 10 chickens – before May 2004 the samples consisted of 10 pooled cloacal swabs) for the detection of *Campylobacter* by culture.

4.5.2 Intervention data

Some of the intervention papers or datasets reviewed and entered into the spreadsheet of possible inputs for the model were of Norwegian origin. Some of the described interventions are so far only applied in Norway or Denmark specifically against *Campylobacter*, e.g. sorting of positive and negative flocks before slaughter, decontamination by use of steam-ultrasound (Denmark) and deep-freezing (Denmark/Norway).

Data on application of the different interventions were collected from the veterinarians/health personell working for the companies Nortura and Norsk Kylling A/S. The data are owned by farmers delivering to Nortura and Norsk Kylling A/S.

4.5.3 Human incidence data

Human incidence data (case – date and place of onset – age of case) based on culturing from 2008 were obtained from the Norwegian Institute of Public Health. A questionnaire is filled in for each case where; campylobacteriosis is suspected and a sample is submitted to the laboratory. Underreporting is still regarded to be a problem. The data are owned by the Norwegian Public Health authorities, provided to the ECDC and consequently summarized in the *Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union*.

4.6 Data from Portugal

4.6.1 *Campylobacter* prevalence or concentration data

Campylobacter prevalence and concentration data were obtained by the Faculty of Veterinary Medicine (FVM). The data include the results of *Campylobacter* diagnosis in several flocks based on the collection of caecum (n=10), neck skin (n=5) and chicken breasts (n=4) from the same flock. The study was performed by FMV in one abattoir. The database includes data on the production system, the duration of slaughter and rejections. The data were obtained under the project SafeFood Era.

4.6.2 Intervention data

This database contains the description of practices in broilers production, slaughter and processing in Portugal.

Sources:

- Data on production was collected through a questionnaire prepared by FMV and posed to veterinarian's assistant in poultry production farms.
- Data on slaughter and meat processing was collected through a questionnaire prepared by FMV and posed to veterinarian inspectors working for the National Veterinary Authority (DGV), through the support of the central services. Questionnaires were collected by the central authority and sent to FMV for analysis.

4.6.3 Human incidence data

This database refers to strains of *Campylobacter* received at the National Health Institute, which is the national reference laboratory in Portugal. The strains were submitted in 2009 by several hospitals. Not all hospitals with bacteriology lab are presently sending samples, only those participating in this *Campylobacter* surveillance program. Most hospitals in the database sent all the isolated strains. From the period of 1-1-2009 to 31-12-2009, 150 strains were isolated. These reported cases are compared, by FMV, with the population of the region of influence of each hospital sending the strains, around 3,954 thousand people (National Statistical Institute).

5 Model Development

5.1 The challenge of modeling campylobacteriosis risk

Campylobacteriosis remains one of the most frequently reported foodborne infections in Europe, particularly from the *Campylobacter jejuni* and *Campylobacter coli* species. *Campylobacter* bacteria are widely distributed in animals such as poultry, cattle, pigs, wild birds and wild mammals. *Campylobacter* are fragile – they cannot tolerate desiccation, and are readily killed by high levels of oxygen. They do not grow in normal atmospheric oxygen levels and are thermophilic, meaning that they require the fairly high temperatures observed in a bird's intestine to be able to grow. Thus, *Campylobacter* do not readily establish reservoirs outside their host animal, which means that human exposure to the bacteria resulting in infection and disease will usually be a random 'rare' event independent of others getting infected. The survival and growth characteristics of *Campylobacter* result therefore in mostly sporadic cases of foodborne infections in humans rather than outbreaks.

The sporadic nature of campylobacteriosis cases, together with the commonly accepted significance of cross-contamination as the most relevant exposure pathway related to poultry, make it much more difficult to trace back the original source of exposure to the *Campylobacter*. Highly discriminatory genotyping methods may be useful in determining the original source of the *Campylobacter* but would require the collection and genotyping of a very large number of samples across the EU. The sporadic nature of campylobacteriosis also makes it very difficult to collect epidemiological data to evaluate a dose-response relationship.

Campylobacteriosis risk assessment modeling is therefore quite challenging. The available experimental dose-response data are few resulting in fitted mathematical dose-response models carrying a great deal of statistical uncertainty about their estimated parameters (see Figure 1) – particularly at low doses, and these experimental data of Black *et al* (1988). were conducted with just two strains of *C. jejuni* (A3249 and 81-176). The strains were collected from patients in two separate outbreaks; strain 81-176 had a much higher attack rate in its outbreak and produced an infection rate of 100% across all experimental doses so provided little additional dose-response information. Put together, this means that using only these data to construct a dose-response relationship excludes the possible effect of variability in pathogenicity of the *Campylobacter* population to which humans are exposed. The experiments were performed on healthy young adults which introduces the additional model (assumption) uncertainty of applying these results to the wider human population (the young and old, different ethnic sub-populations, and the immuno-compromised) and variable ingestion events (the Black *et al.* (1988) experiment provided the ingested dose in milk to participants who had not eaten for 90 minutes before or after the dose was administered). Moreover, there is some evidence that exposure to low doses of *Campylobacter* causes short-term partial immunity to infection, further confounding the interpretation of a dose-response relationship.

Nauta *et al.* (2009) plotted various dose-response models (Figure 2) that have been used in *Campylobacter* risk assessments, noting that the graph “clearly illustrates the uncertainty about the “true” dose-response relationship for *Campylobacter*”. The lack of certainty for low dose is particularly relevant since intervention strategies are usually aimed at reducing the microbial load on the food products and therefore on the ingested dose: the smaller this becomes, the proportionally greater the uncertainty associated with the probability of infection.

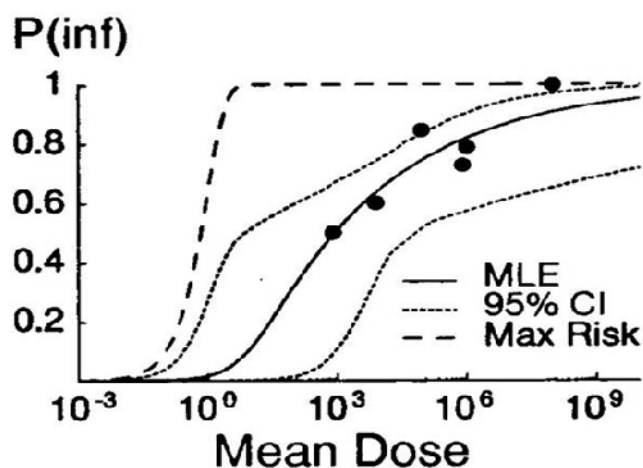


Figure 1 Beta-Poisson Dose-Response Model with confidence interval (Teunis and Havelaar, 2000)

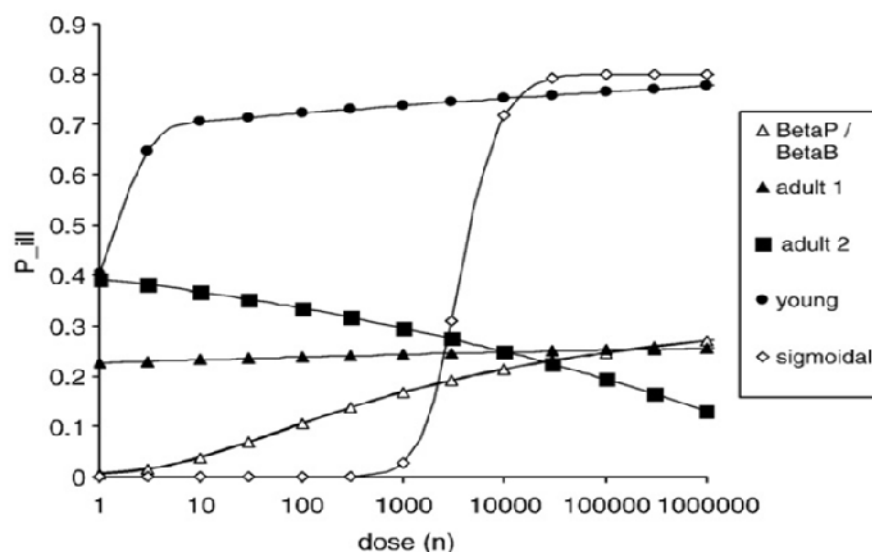


Figure 2 Dose-Response Model in *Campylobacter* risk assessments (Nauta et al., 2009)

Case-control studies from the US CDC among others have demonstrated a statistical relationship between the risk of contracting campylobacteriosis and the consumption or handling of poultry meat, alongside several other factors (e.g. Friedman et al., 2002). It remains difficult to estimate how much these other factors (for example, the presence of pets in the household, the consumption of untreated water, or visiting a farm) are also influenced by the levels of *Campylobacter* in poultry because of contamination of feed and water, cross-contamination, and the inappropriate handling of food (for example, feeding raw or undercooked food to the family pet, using the same utensils in a kitchen for chicken preparation and uncooked foods, or just failing to wash one's hands sufficiently well).

The role of poultry in *Campylobacter* infections has been statistically demonstrated by analyzing the effect of the unfortunate Belgian dioxin crisis in 1998. During that event the incidence of campylobacteriosis dropped together with the withdrawal of poultry meat from the market for several

weeks (Vellinga and Van Loock, 2002). Still, it remains difficult to estimate the total rate of campylobacteriosis due to chicken because Belgian chicken was partially replaced by foreign-sourced chicken which may have had a different level of contamination. Other meats that consumers purchased in place of chicken may have carried *Campylobacter*. Some infrequent outbreak data have also confirmed the role of chicken (e.g. Jiménez M, 2005) – both via suspected cross-contamination from raw chicken to other foods). The Dioxin crisis did demonstrate very well that the removal of an important source of *Campylobacter* results in a precipitous decrease in the rate of infections, which is advantageous in finding simpler risk assessments designs.

In summary, it is critically important to the design of a successful, robust risk assessment for *Campylobacter* that one acknowledges the paucity of data and the complexity of the routes of exposure. The vulnerability of the assessment of a possible intervention to model and statistical uncertainty must be transparent and consistent, and minimized through the design of the risk assessment, if one is to produce results that are of real value to the risk manager. It is this need that drives the approach that we took in developing the model.

5.2 Our modeling approach

We have built a model that focuses on intervention strategies that the decision-makers wish to consider. The EFSA BIOHAZ WG has provided a list of interventions to consider and ranked each intervention into three categories reflecting the importance that they place on their inventions being evaluated: essential; highly desirable; and desirable.

The focus of the model has been to evaluate the human health benefit of these interventions in the most robust fashion possible. In order to avoid the large uncertainty in the estimate of health risk that can occur by propagating the estimated prevalence and level of contamination through a dose-response model, we used the following general approach:

1. Built a model reflecting the current situation for the particular EU country, with a focus on the area in which the selected intervention strategy would apply. The model incorporates inter-individual variability and stochastic behavior;
2. Calibrated this model to levels of observed rates of campylobacteriosis that can be directly or indirectly attributable to poultry using two different dose-response functions;
3. Modeled the effect of the intervention on microbial load and/or between flock prevalence (i.e. the fraction of flocks that are infected) and/or within-flock prevalence (i.e. the fraction of birds within an infected flock that are infected, or post slaughter the fraction of carcasses in a batch from a single flock, which might alternatively be called ‘within-batch prevalence’ at that stage) and evaluated the relative change in the predicted human health burden using (in stochastic mode) the same sampled values from uncertainty distributions as 1, or in deterministic mode using the same best estimate;
4. In stochastic mode, repeat stages 1 to 3 with new samples from the uncertainty distributions of the model parameters. In deterministic mode, the best estimate is used to give a single-valued output.

The net result of this approach has been to ensure that the impact of statistical and model uncertainty does not disguise the estimated change in human health impact. Each dose-response model accounts for the non-linear nature of the dose effect, and performing a comparative analysis helps minimize the impact that not knowing the true dose-response relationship has on the estimated human health impact. A sensitivity analysis described in Section 6.4.1 illustrates that the model’s outputs are relatively insensitive to the choice of dose-response function.

Our modeling approach maintains consistency with the four components which have been defined for microbial risk assessment in food by the *Codex Alimentarius* Commission: (i) hazard identification; (ii) exposure assessment; (iii) hazard characterization; and (iv) risk characterization.

5.3 Description of the model mathematics

The model developed for this contract uses probability mathematics to determine the distribution of microbial load on a bird or carcass through the various stages from shed to chilled carcass, together with the prevalence of *Campylobacter*-contaminated flocks and the within-flock prevalence for those contaminated flocks.

The distribution of the level of microbial contamination is described in the model by the normalized central moments of the log 10 distribution of colony forming units (cfus) per chicken or carcass, namely:

- Mean
- Variance
- Skewness
- Kurtosis (where a Normal distribution has a kurtosis of 3)

The mathematics are based on raw moments. The formulae for conversion between raw and centralized moments and back are described in Annex 1, Section A. The effect of interventions on microbial contamination is modeled in terms of the log (always in base 10) change that the intervention has on the contamination. The equations of Annex 1, Section B provide the mathematics that relate the log load moments before and after an intervention. The purpose of using the moments to describe log contamination levels and the log effect of interventions is to be free from having to make assumptions about the distributional form at each stage. This is particularly useful, for example, when one only partially applies an intervention within a country since the effect of that intervention is then bimodal which cannot be well-described by any single distribution. The equations of Annex 1, Section C describe how the effect of a partial application of an intervention is determined.

Changes in flock prevalence and within-flock prevalence are based on logit functions. The equations of Annex 1, Section E provide the equations.

The model is thus able to determine, through calculation, the normalized central moments of the level of contamination of a carcass post chilling and the fraction of carcasses that will be contaminated (equal to the product of the flock prevalence and within-flock prevalence).

The model then fits a distribution to the first three normalized central moments of the contamination log level. If the skewness is extremely small (absolute value < 0.01), it assumes a Normal distribution (equivalent to a lognormal distribution of actual number of bacteria) using the mean and variance. Most commonly, the skewness is positive (a longer right tail), in which case a shifted-Gamma distribution is matched to the mean, variance and skewness. If the skewness is negative, an inverted shifted-Gamma distribution is used. Annex 1, Section C provides the equations. The purpose of converting the calculated moment to a probability distribution is that one can then assign a probability density $f(x)$ and cumulative probability $F(x)$ to any specific log level of contamination x , which are then used in the dose-response component of the model.

Kurtosis values are not actually used within the model. They are a part of the output of the VoseNBootMoments array function and so needed to be generated. However, they do provide an appreciation of the form of the distribution that is being generated: values around 3 are consistent with a Normal distribution, values much larger than 3 represent a distribution that is more peaked than a Normal,

while values less than 3 are flatter than a Normal. The Gamma distribution always has a kurtosis greater than 3.

5.3.1 Dose-response functions

The dose-response component of the model relates the distribution of the number of *Campylobacter* cfu on contaminated carcasses (both indoor and outdoor) to the true number of cases of campylobacteriosis in a country attributable to poultry. All the pathways between the point after chilling of the carcass and the exposure of a random person to *Campylobacter* from a contaminated carcass are implicitly contained within this function. The dose-response component also implicitly incorporates the variation between individuals in their susceptibility, the amount of food they eat, the way they prepare and handle the food, as well as pathways that provide an indirect exposure to poultry-derived *Campylobacter*. Two dose-response functions are used, and another that combines these two. Each function requires a single parameter that is estimated within the model by varying its value until it predicts the current true number of cases of campylobacteriosis using the current prevalence and load estimates. It is only possible to use a single parameter function because only one data point is available to estimate the parameter.

The two different functions are used to test how sensitive the model results are to the choice of dose-response function. The two dose-response functions are:

1. Simple exponential
2. Modified Beta-Poisson multiplied

A visual explanation of the general approach to the exposure calculation is provided in Figure 3 using the *Simple exponential* as an example. The combined function allows one to perform a statistical analysis of the sensitivity of the output to the choice of dose-response function.

5.3.1.1 The Simple Exponential dose-response function

The *simple exponential* dose-response takes the form:

$$P(\text{illness}) = 1 - \exp(-rx) \quad \text{Equation 1}$$

where r is a dose-response parameter and x is log10 of the ingested dose. The model runs two sets of parallel calculations for indoor and outdoor-reared poultry and then aggregates the results to estimate the number of observed infections.

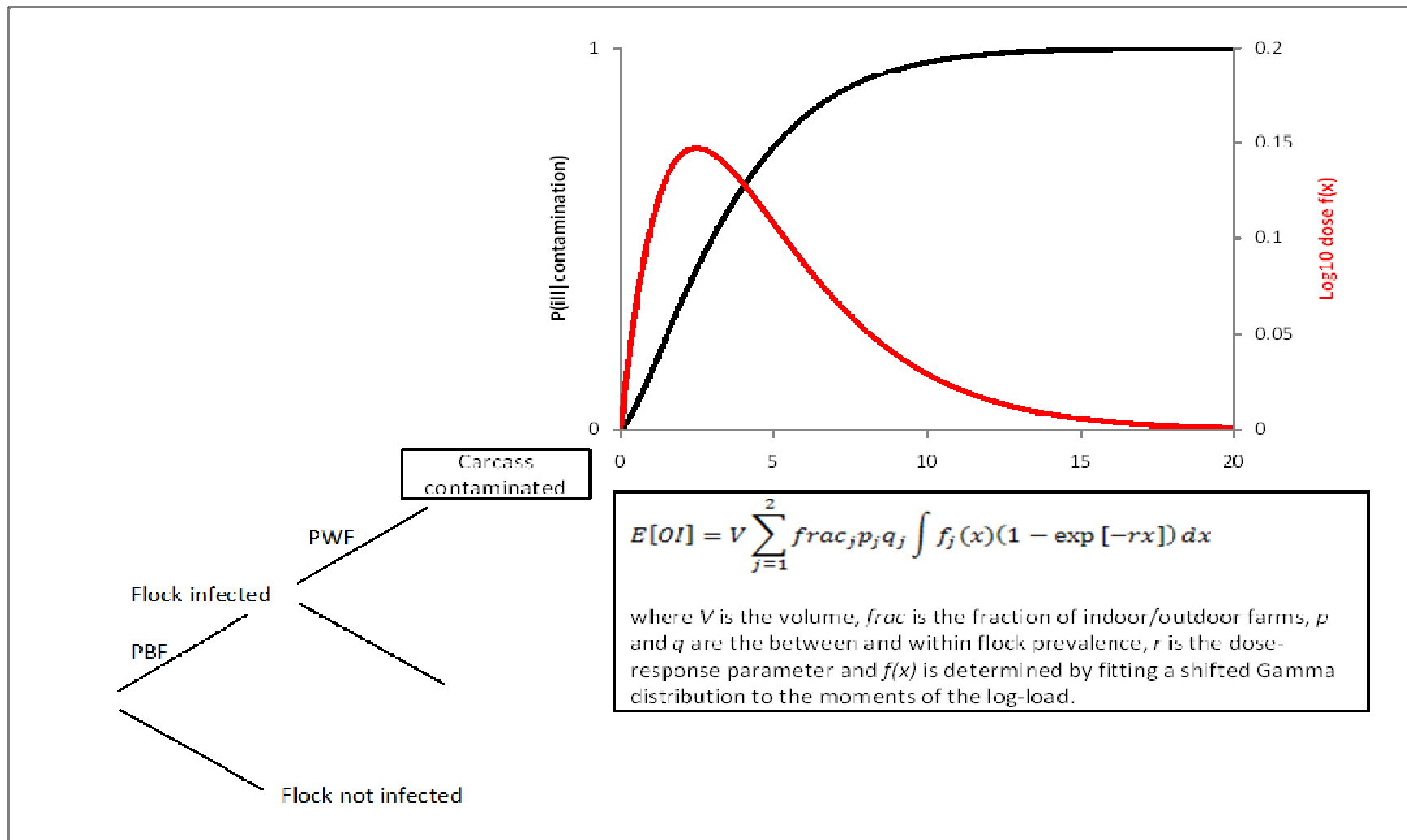


Figure 3 Illustration of the principle behind the model. From the left, the event tree considers the probability that a flock of type j is infected (between flock prevalence, PBF). Then it considers the probability that a random bird from within that flock is infected, and will remain contaminated up to the point where its carcass has been chilled. Finally, the model uses the fitted distribution of microbial load (in red) together with the dose-response curve in an integral calculation to estimate the probability that a person will become an identified campylobacteriosis case by exposure to *Campylobacter* from this carcass.

The expected number of observable illnesses (OI) is determined as follows:

$$E[OI] = V \sum_{j=1}^2 \text{frac}_j p_j q_j \int_0^{\infty} f_j(x) (1 - \exp[-rx]) dx \quad \text{Equation 2}$$

where:

V = the number of poultry consumed per year from the country in question

$j = 1$: Indoor poultry; $j=2$: Outdoor poultry

frac_j = the fraction of poultry raised in condition j . $\text{frac}_1 + \text{frac}_2 = 1$

p_j = flock prevalence

q_j = within flock prevalence

f_j = the density of the log level of contamination in a carcass post-chilling

x = the log level of contamination in a carcass post-chilling

r is the dose-response parameter

The r parameter is estimated within the model using the VBA function VoseDRExpPopulationPairedFitP, where r is varied using a solving algorithm until, using the current state in Equation 2, the variable $E[OI]$ matches the current true (i.e. corrected for under-reporting) number of illnesses attributable to poultry.

Interpretation: The r value is a multiplier on the distribution of log10 load. Estimates of r -values within the model are typically of the order of 10^{-5} to 10^{-6} , whereas the log10 dose is typically of the order of 0 to 10. The product rx is therefore orders of magnitude below 1, producing a curve similar to Figure 4. This means that this dose-response function is approximately linear in log dose.

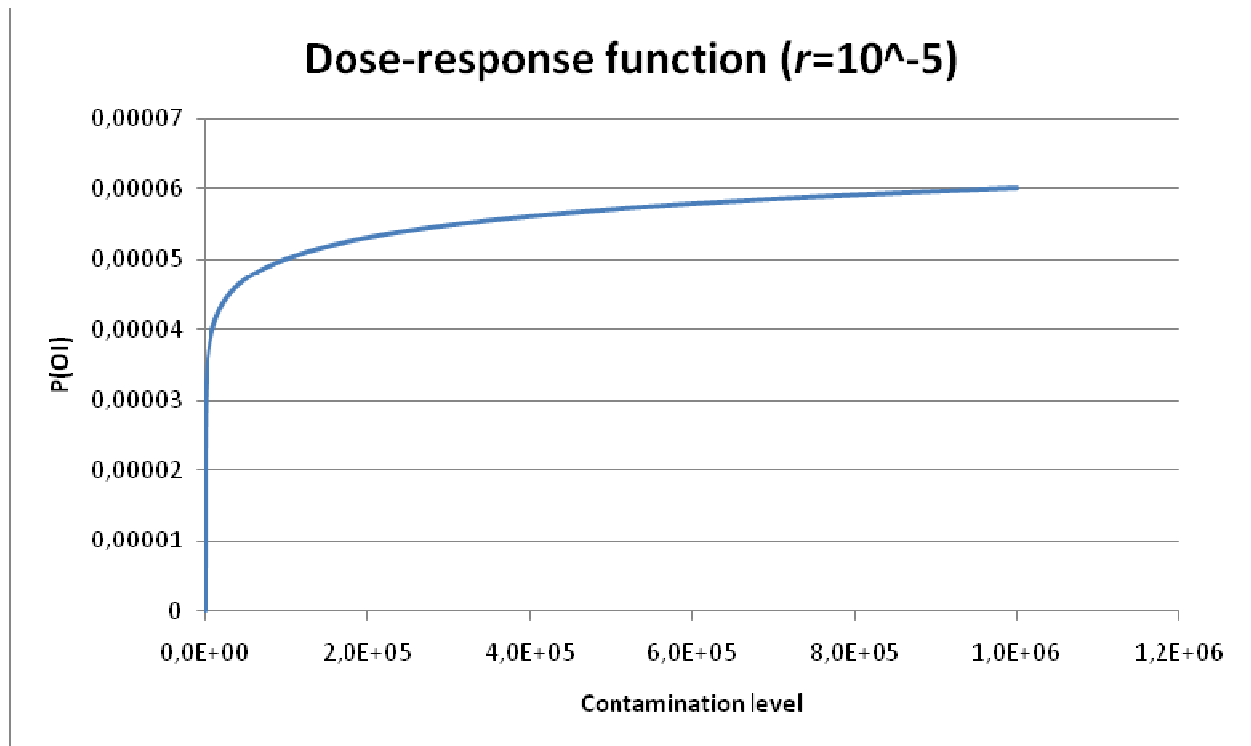


Figure 4 Typical fitted population dose-response curve

Error generation: In occasional extreme scenarios the fitting algorithm in the VoseDRExpPopulationPairedFitP will reach an overflow error during its integration routine. The

function then returns ‘Overflow error’ to inform the user of the reason it cannot compute a result rather than hang the model or return #VALUE! that Excel would normally provide. Further calculations dependent on the estimate of the dose-response parameter also return the message ‘Overflow error’ when this occurs.

5.3.1.2 Modified Beta-Poisson dose-response function

The standard *Beta-Poisson* dose-response takes the form:

$$P(\text{illness}) = 1 - \left(1 + \frac{x}{\beta}\right)^{-\alpha} \quad \text{Equation 3}$$

where α , β are dose-response parameters and take the usual values 0.145 and 7.589 derived by fitting to the Black *et al* (1988) data and x is the actual number of cfu ingested dose (Figure 5).

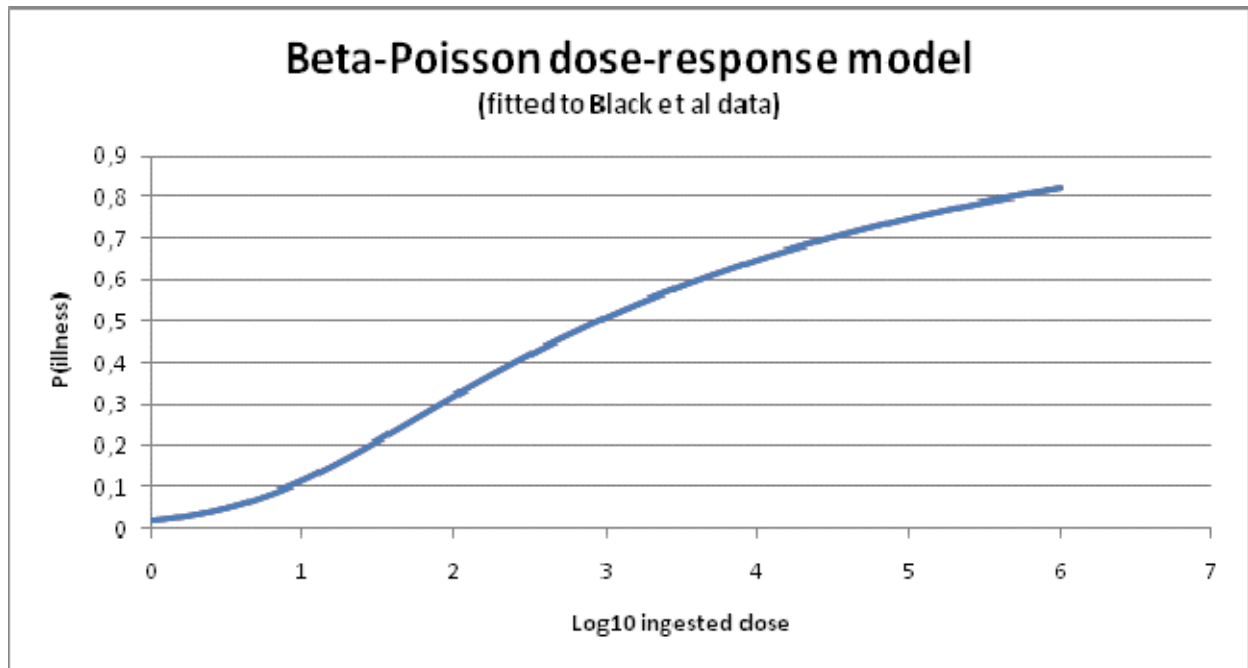


Figure 5 Fitted Beta-Poisson dose-response model ($\alpha = 0.145$, $\beta = 7.589$)

For this version of the Beta-Poisson model, the dose-response function is multiplied by some probability b . In a similar manner to Equation 2, and using the dose-response function of Equation 3, we then have:

$$E[OI] = V \sum_{j=1}^2 \text{frac}_j p_j q_j \int_0^{\infty} f_j(x) b \left(1 - \left(1 + \frac{x}{\beta}\right)^{-\alpha}\right) dx \quad \text{Equation 4}$$

The b parameter is estimated directly within the model in Sheet ‘Dose-Response’ so that the variable $E[OI]$ matches the current true (i.e. corrected for under-reporting and source attribution) number of human illnesses. The integral within Equation 4 is not always algebraically solvable. The model therefore makes an approximation to this integral by splitting the log dose into 0.2 increments over the range [0,17].

Interpretation: The b value is a probability multiplier. It can be interpreted as leaving the dose distribution post-chilling unaffected and assigns a probability that this dose will reach the consumer. It can also be interpreted as the expected number of illnesses that would occur from a post-chilled carcass carrying the load distribution x .

5.3.1.3 Combined dose response functions

A third dose-response function combines the results from the two dose-response functions described above. It does this by randomly selecting between the model's estimates for reduction in human cases by each dose-response function using a Discrete distribution. It selects each result in proportion to the weights provided by the user in the sheet 'Inputs and Control'. The default weightings are {1,1}, i.e. the two dose-response functions are assigned equal credibility. Values generated by the Discrete distribution are automatically stored in a simulation run. This allows the automatic assessment of the sensitivity of the output to the selection of dose-response function when reviewing the results from this combined function. The selection appears as a variable named 'Model selection switch' in the sensitivity analysis graphs (see Section 6.4.2, Figure 12). The only exception is that for some extreme scenarios the *Simple Exponential* dose response function will not find a solution in which case the combined dose-response function will select only the result from the *modified Beta-Poisson* dose response function.

5.3.1.4 Other dose-response functions considered

A Beta-Poisson dose-response function with shifted log-load was tested for use in the model. For this version of the Beta-Poisson model, the log10 dose distribution is shifted by some constant k , or equivalently one can say that the dose distribution is divided by a constant 10^k . In a similar manner to Equation 2, and using the dose-response function of Equation 3, we then have:

$$E[OI] = V \sum_{j=1}^2 \text{frac}_j p_j q_j \int_0^{\infty} f_j(x) \left(1 - \left(1 + \frac{x}{10^k \beta}\right)^{-\alpha}\right) dx$$

In most modeled scenarios this approach yielded similar results to the other dose-response functions. However, when the fitted k parameter was large, the load distribution was shifted so far to the left that the exposure estimate (integrated from 0 onwards) became extremely sensitive to the shape of the far-right tail of the load distribution, which sometimes produced illogical results like a many-fold increase in incidence with a significant reduction in the post-chilling mean log load. In conjunction with the difficulty in interpretation of the k factor, it was decided not to include this dose-response function.

A linear dose-response function was also considered, i.e. $P(ill) = c\bar{x}$, where \bar{x} is the mean cfus post chilling. Since load distributions can have very long right tails, \bar{x} is dominated by the shape of the right of the log load distribution. Small differences in that tail can produce radically different and unintuitive human incidence rate estimates. Moreover, for some combinations of α and β , $10^{\Gamma(\alpha, \beta)}$ distributions fitted to the log load post chilling have an undefined or infinite mean making the calculation of \bar{x} impossible.

This dose-response function was therefore not included in the model.

5.3.1.5 Critical review of the dose-response functions

EFSA commissioned a review of the model presented in this report by Maarten Nauta from the WG. Dr Nauta's expressed considerable concern that the model's results could actually be very sensitive to the dose-response model used. To illustrate this, he applied a dose-response function from his own work which he calls the 'Classic+' model.

5.4 Normalization of the Model to the available data

The model has two halves. The first half represents the situation in the MS as it is currently known to be. This half of the model is the one which is normalized to the observed data. The second half of the model provides the user with the ability to investigate possible changes to the set of interventions that are applied in that MS. This second half uses the values that have been normalized in the first half of the model to be able to make a direct comparison and thus determine the fractional change in human health impact that would occur with a new set of interventions.

The model has three data sets against which it has to normalize:

1. The observed number of identified human cases of campylobacteriosis that are attributed to *Campylobacter* from poultry from the Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union
2. The EFSA baseline survey data on prevalence of *Campylobacter*-infected flocks in each MS (pooled samples of ceaca)
3. The EFSA baseline survey data on levels of contamination on carcasses post chiller (skin samples).

Data set 1 is for most member states a significantly small fraction of suspected (observable) cases of campylobacteriosis within the country. How underreporting is accounted for is described in Section 5.5.

Data set 2 provides good data on whether each randomly selected flock is contaminated. Simulation studies we have done using data produced by Lelystad and explained in Katsma et al (2007) on levels of *Campylobacter* infections in poultry in infected flocks show that the test has almost 100% probability of a contaminated sample using this method if the flock is infected, even if the flock has only recently become infected. However, there may be some underestimation due to the test sensitivity itself. EFSA will be re-evaluating the between-flock prevalence estimates incorporating test sensitivity. These new values can be input in sheet 'Inputs and Control'. The current deterministic values used for between flock prevalence for each MS are determined as the ratio of the number of flocks tested in that MS s that had at least one of a ceacal or skin sample positive to the total number of flocks tested n . In stochastic mode, the model uses Beta($s+1$, $n-s+1$).

Data set 3 provides counts of bacteria from pooling skin samples from the neck and breast. They do not give actual estimates of the number of *Campylobacter* on each tested carcass. This model assumes that the enumerated CFU values are however proportional to the actual level of CFU contamination on the carcass as a whole. The WG advised that a multiplying factor of 100 (2 logs) should be applied to provide the number of bacteria on the carcass. This value can be altered if required in sheet 'Inputs and Control'.

The model also needs to normalize to the within-flock prevalence for infected flocks. No systematic data are available for this by MS so it was assumed that an infected flock, for which no interventions to reduce within-flock prevalence were applied, would have a 100% within-flock prevalence.

The model shows with cells with a red background (Figure 6) the anchor points from which the model performs backward and forward calculations. In brief:

1. The dose-response parameter is numerically solved to produce a value in that matches the observed number of identified cases;
2. The EFSA flock prevalence data is entered at line 'Slaughter Plant' in sheet 'Country'. Bearing in mind the interventions that have already been applied within that MS, a 'naïve' flock prevalence is back-calculated to determine what this would be in a counterfactual world in which no interventions had been applied. Forward calculations alter the flock prevalence (or equivalently batch prevalence once a flock has entered the slaughter process) to account for interventions that are applied after the poultry leave the shed; and
3. The EFSA post-chilling contamination level data is entered at line 'After Chiller' in sheet 'Country'. Bearing in mind the interventions that have already been applied within that MS, a 'naïve' contamination level (or equivalently microbial load level within the live chicken on the farm) is back-calculated to determine what this would be in a counterfactual world in which no interventions had been applied. Forward calculations alter the contamination levels to account for interventions that are applied after chilling.

Since these three data sets provide information at different points along the farm to human illness continuum, it has by necessity been assumed that the three anchoring points above can be analyzed

independently. Thus, the model first estimates the microbial load and prevalence levels post-processing of the carcasses, and then matches these estimates to the recorded human illness rates by numerically solving for the dose-response parameter.

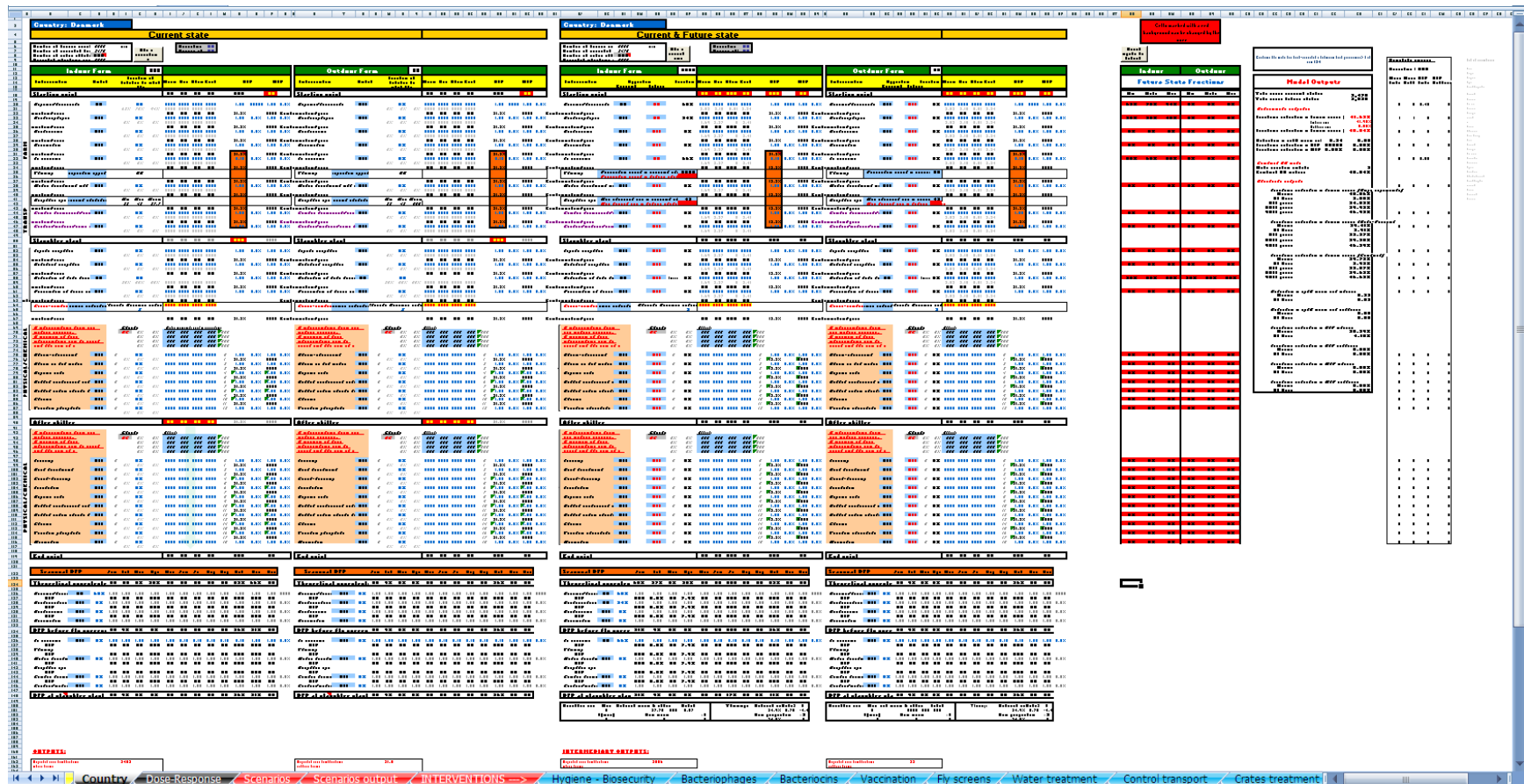


Figure 6 Overview of the ‘Country’ sheet of the model when all calculations are revealed. The two large tables at left are calculations for the current and future states, showing points from which calculations are anchored (yellow text on a red background). The temporal flow is top to bottom. Thus, in the left most table of the model (the current state), the directions of forward calculations flow down from the red cells and the directions of backward calculations flow up from the red cells. In the complimentary right hand table (the counterfactual world with alternative intervention strategies) the flow is downwards from the top where the naïve levels of load and prevalence are shown at the farm. The two red cells in the top left corner show the number of reported cases attributable to poultry – the anchor point for estimating the dose-response parameters. The red table to the right allows the user to input intervention data, and the far right table provides the model’s results.

The model then replicates the analysis for a counterfactual world starting with the estimated ‘naïve’ values for microbial levels in the live chicken, flock and within-flock prevalence. The user can then select the interventions that should be applied, and the model provides forward calculations along the farm-to-consumer continuum of the effect of these interventions on contamination and prevalence levels. Finally the parameter-value from the dose response function estimated from the first half of the model is used to determine the expected level of identified cases. If the user inputs the same interventions and level of use as shown in the first half of the model, the model will naturally return a zero level of change in human illness rates.

5.4.1 Accounting for seasonality in between flock prevalence

EFSA baseline data show very marked patterns in between flock prevalence over the course of the year. Using these data, we have determined the monthly between flock prevalence for each country, reported in sheet Inputs & Control. Where no data are available for a specific country, EU averages have been used. Where data are missing for a specific month, the average of the previous and next month is used. These gap-filling alterations are highlighted within the spreadsheet.

Figure 7 shows the observed monthly variations in between-flock prevalence for several representative MS.

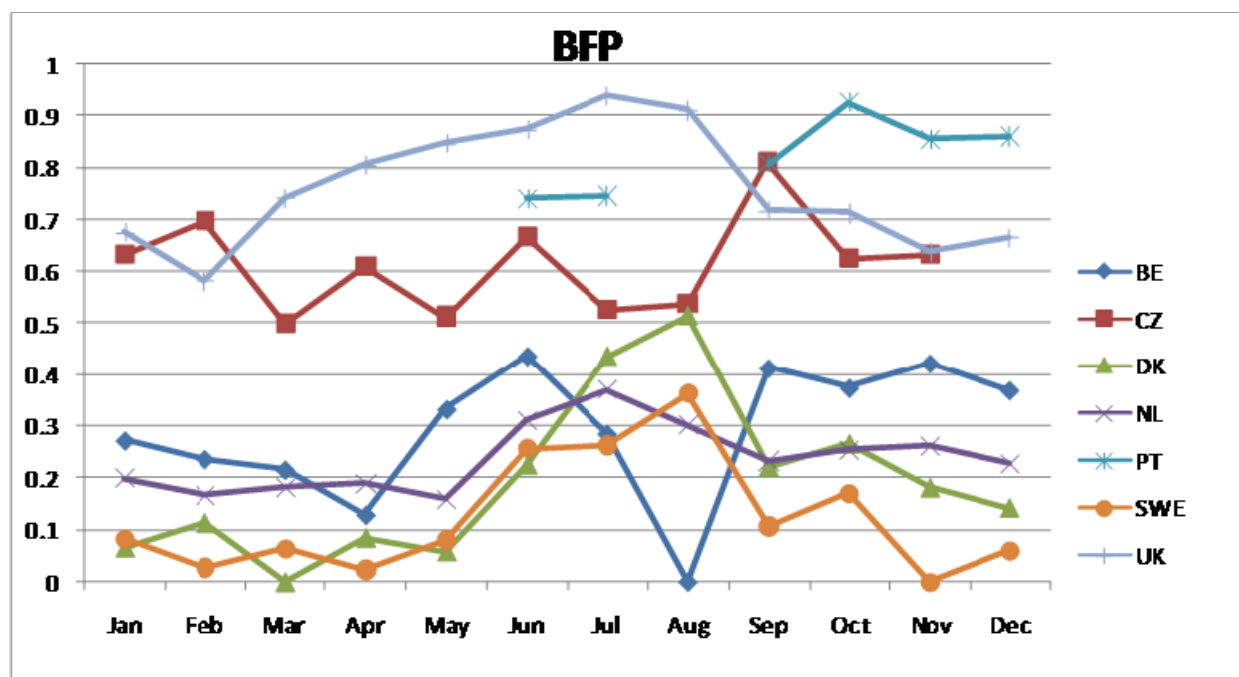


Figure 7 Observed monthly variations in between-flock prevalence for several representative MS.

It was considered important to account for this seasonal variation by month because one of the identified interventions (fly screens) would only be applied or have any effect in certain months of the year and would affect the probability that *Campylobacter* enters a shed - and thus the between-flock prevalence. In Sheet ‘Country’ one can enter the start and finish months for which fly screens would be in place and effective, and the model automatically adjusts the between flock prevalence for each month as applicable. Assuming that poultry production remains constant for each month of the year, the annual between flock prevalence is calculated as the average of the monthly between flock prevalence values.

5.5 Estimation of the number of human cases of campylobacteriosis

The model is normalized to the estimated number of human cases of campylobacteriosis attributable to poultry in each country $E[OI]$ in Equations 1-4. This variable is estimated as follows:

$$E[OI] = \frac{Ca}{u}$$

where:

C = the number of observed cases in that country

a = is the fraction of cases that are attributable to poultry

u = the under-reporting factor for that country

The number of reported cases C is drawn from the ECDC *Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union*. The data appear in sheet Inputs & Control. There are no consistent data available for the fraction of human cases of campylobacteriosis that are attributable to poultry across MS, so the model uses a placeholder value of 30%. Separate entries have been used for each country in sheet Inputs & Control and can be altered as the user wishes. The under-reporting factor u accounts for the difference between the number of actual cases of campylobacteriosis in a country and the number that are reported. This will vary between MS according to the frequency with which people suffering from campylobacteriosis will seek medical intervention, the degree to which cultures are taken, the degree to which these results are collected and officially reported, etc. The reported incidence rates vary considerably strongly supporting the notion that under-reporting rates vary significantly between MS. The under-reporting factors used in the model (shown in [Table 1](#)) were provided by the chairman of the WG, Arie Havelaar, and based on de Jong and Ekdahl (2008). Note that some under-reporting factors exceed 100%, which would mean that these countries report more campylobacteriosis cases than actually occur. The under-reporting factor has negligible effect for these countries because the data show reasonable levels of incidence, and the under-reporting factor only has some level of influence on the model results when the observed incidence is extremely small.

Table I Pedigree matrix clarifying the score for the 5 criteria for assumption assessment	
Country	Under-reporting rate
Austria	11.6%
Belgium	4.2%
Bulgaria	0.0%
Cyprus	0.2%
Czech Republic	7.6%
Denmark	30.0%
Estonia	2.3%
Finland	176.4%
France	0.3%
Germany	29.8%
Greece	0.3%
Hungary	3.9%
Ireland	2.9%
Italy	0.1%
Lithuania	4.5%
Luxembourg	17.1%
Malta	1.0%
Poland	0.0%
Portugal	0.0%
Romania	0.0%
Slovakia	2.3%
Slovenia	2.1%
Spain	0.3%
Sweden	175.9%
The Netherlands	4.2%
United Kingdom	11.4%
Norway	42.5%
Switzerland	16.2%

5.6 Mutually exclusive interventions

The set of physical and chemical interventions are all mutually exclusive, meaning that two physical/chemical interventions will not be applied on chickens from the same farm. However, it is possible that within a country, chickens from X percent of the farms will undergo chemical intervention 1 and Y percent of the farms will have chemical intervention 2. This means that we have to find a function that calculates the central moments resulting from subtracting fractions of different set of moments (representing the interventions) from another set of moments (representing the load before applying the mutually exclusive interventions). If A represents the log load before applying the interventions and $I1$ and $I2$ are the log loads corresponding to intervention 1 and 2, then the log load B after applying the interventions is equal to:

$$B = A - X * I1 - Y * I2,$$

where X and Y are the fractions of farms applying intervention 1 and 2 respectively. Since we cannot have it that 80% of the farms apply intervention $I1$ and 40% of the farms apply intervention $I2$ (we said all interventions were mutually exclusive), the sum of X and Y cannot exceed 1.

It is possible that more than two physical/chemical interventions are applied on different percentages of farms within a country, but given the exponentially increasing complexity of the mathematics behind the calculation of the higher order moments for more than two interventions, we have chosen to limit the number to four. Looking at the physical/chemical interventions currently applied in Belgium, Denmark, Norway, Greece or Portugal, we feel that this is sufficient. The equations of Annex 1, Section C describe how the effect of the application of mutually exclusive interventions is determined.

In the model, this means that the user can switch on four of the seven physical/chemical interventions *before chiller* and four of the ten physical/chemical interventions *after chiller* (see pink colored area in Figure 8).

Contaminations <pre></pre>										Contaminations <pre></pre>									
67										67									
68										68									
69										69									
70										70									
71										71									
72										72									
73										73									
74										74									
75	Stream-ultrasound	OFF	OFF	0	0x	2.50	0.00	0.00	3.00	0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
76	Stream or hot water	OFF	OFF	0	0x	1.84	0.98	0.00	3.00	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
77	Organic acids	OFF	OFF	0	0x	1.70	0.00	0.00	3.00	4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
78	Acidified electrolyzed oxidizing w.	OFF	OFF	0	0x	0.00	0.00	0.00	3.00	6	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
79	Acidified sodium chlorite (ACS)	OFF	OFF	0	0x	0.00	0.00	0.00	3.00	7	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
80	Chlorine	OFF	OFF	0	0x	0.00	0.00	0.00	3.00	8	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
81	Tetrasodium phosphate	OFF	OFF	0	0x	0.00	0.00	0.00	3.00	12	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
82										82									
83										83									
84										84									
85										85									
86										86									
87										87									
88										88									
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92										92									
93										93									
94										94									
95										95									
96										96									
97										97									
98	Freezing	ON	ON	1	0x	0.00	0.00	0.00	3.00	0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
99	Heat treatment	ON	ON	2	0x	0.00	0.00	0.00	3.00	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
100	Cross-freezing	ON	ON	3	5x	0.00	0.00	0.00	3.00	4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
101	Irradiation	OFF	OFF	0	0x	4.70	0.00	0.00	3.00	6	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
102	Organic acids	OFF	OFF	0	0x	1.70	0.00	0.00	3.00	7	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
103	Acidified electrolyzed oxidizing w.	OFF	OFF	0	0x	0.00	0.00	0.00	3.00	8	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
104	Acidified sodium chlorite (ACS)	OFF	OFF	0	0x	0.00	0.00	0.00	3.00	12	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
105	Chlorine	OFF	OFF	0	0x	0.00	0.00	0.00	3.00	13	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
106	Tetrasodium phosphate	OFF	OFF	0	0x	0.00	0.00	0.00	3.00	17	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
107	Minimization	OFF	OFF	0	0x	0.00	0.00	0.00	3.00	18	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Figure 8 Illustration of where with the ‘Country’ sheet of the model the user can switch the mutually exclusive interventions on and off and change the fraction of application

The model groups the moments of the four (or less) selected interventions (blue-grey background) and then applies the function. Since on the left side of the model (the current state), the direction of calculations flows from the “After chiller” anchor point onwards, we need one function that calculates the moments before we applied the first set of mutually exclusive interventions (backward calculation) as well as a function that calculates the moments after applying the second set of mutually exclusive interventions (forward calculation). In the right side of the model (the counterfactual world with alternative intervention strategies) the flow is downwards from the top so we only need a function that

calculates the set of moments after applying a set of mutually exclusive interventions. Equations for these two functions can be found in Annex 1, Section C.

5.7 Non-standard modeling of interventions

Most of the interventions modelled use the mathematics described in Annex to alter the log load moments of within or between flock prevalence. However, there are some interventions that required modifications to this approach, described here.

5.7.1 Accounting for cross-contamination within a slaughter plant

It is recognized that cross-contamination at the slaughter plant redistributes the *Campylobacter* on carcasses. On its own, i.e. not looking at the effects of removal of bacteria, cross-contamination has the effect of reducing the variance in the load distribution on carcasses. This can be understood intuitively by imagining that a very large number of contacts between carcasses would ultimately lead to all carcasses carrying the same number of bacteria. In other words, the mean number of bacteria is not affected but the variance is reduced.

The effect of cross-contamination is included within the model as a user entered reduction in the variance of the log10 load. By default this has been set to a default value of 2. This can be changed to different values by the user in sheet 'Inputs and Control' for the before and after scenarios separately. It was hoped that the WG would supply a researched value from Maarten Nauta's simulation studies prior to the writing of this report, but the value has not yet become available. The model then alters the log mean and log variance within the slaughter plant such that the mean of the actual cfu is unaffected by the reduction in the log variance. This is based on the principle that a variable X following:

$$\tilde{X} \sim 10^{\text{Normal}(\mu, \sigma)}$$

has a mean of $\exp\left[\mu \ln[10] + \frac{(\sigma \ln[10])^2}{2}\right]$. Thus if one reduces the variance by some amount δ but maintain the same mean, we have:

$$\mu_b + \frac{\sigma_b \ln[10]}{2} = \mu_a + \frac{(\sigma_b - \delta) \ln[10]}{2}$$

where b, a subscripts refer to before and after cross-contamination respectively. Thus, in order to change the log variance but retain the same mean number of bacteria on a carcass, the models makes the correction:

$$\mu_a = \mu_b + \frac{\delta}{2} \ln [10]$$

The model adjusts the log load at each step through the farm to post chilling process in an additive manner (i.e. multiplicative in terms of actual bacterial counts). Thus, it is possible to incorporate this cross-contamination effect anywhere within the slaughter plant section of the model without specifically allocating where the variance reduction occurs, because $a+b+c = a+c+b$, irrespective of whether a, b, c are fixed values or random variables.

5.7.2 Reprocessing of highly focally contaminated carcasses

The WG asked that an intervention be modeled in which the carcasses with the highest x% (specifically 10%) of fecal contamination be ‘reprocessed’ reducing the level of contamination by y logs (specifically 0.9 logs). The model achieves this by splitting the original log load distribution, assuming it to be $\text{Normal}(\mu, \sigma)$, into two parts:

$\text{Normal}(\mu, \sigma)$ bounded with a maximum at $\Phi^{-1}(1 - 100x)\sigma + \mu$

(i.e. bounded at the value for which x% of the distribution lies above)

$\text{Normal}(\mu, \sigma)$ bounded with a minimum at $\Phi^{-1}(1 - 100x)\sigma + \mu$ and then shifted by -y.

Moments for the two distributions are calculated using the ModelRisk VoseMoments function, which performs a numerical integration over the truncated distributions. The new log load mean and variance are then calculated and the reduction in log load mean and variance determined. This is implemented in sheet Detection in the model.

The results cannot be used in the usual way because it is not possible to perform the reverse calculation, i.e. it is impossible to determine what the mean and variance would have been prior to this intervention when one knows only the mean, variance after the intervention, together with x and y. Thus, for this particular intervention, the user is asked to enter the incremental fractional use for which this intervention might be applied. So, for example, if one estimated that the high fecal reprocessing was applied to 10% of slaughtered flocks but now would be applied to 30%, the user would enter 20% (=30%-10%) in Cells AP58 and BG58 in Sheet Country. This is a workaround that in a couple of ways: first, the assumption of lognormality of load may be inaccurate, and secondly the incremental component has a second order error in that it would be applying a reprocessing to distributions of carcasses in which some reprocessing has already occurred. The model thus works best if the reprocessing intervention is currently not implemented at all or very little.

5.7.3 Single interventions with very large log load impact applied to a fraction of flocks

The model assumes at the end of all interventions that the log load can be modeled as a unimodal (single peak) distribution. This is necessary if it is to fit a Normal or Gamma distribution to the resultant log load moments.

A single intervention with a very large log load distribution applied to a fraction f of flocks would naturally produce a bimodal distribution. A ‘very large log load reduction’ would mean that there would be little overlap between the before and after log load distributions. In this situation, the user should model the effect of the intervention as if it was applied to all flocks and then multiply the final estimated reduction in human health incidence by the fraction f .

5.7.4 Hygiene-biosecurity confidence level

The WG asked to use results from Gibbens et al (2001) in the model for the effect of hygiene-biosecurity, but allow them to manually adjust the regression parameter from this paper by adjusting the percentile level. This is implemented in sheet Hygiene-Biosecurity at cell L38. In Figure 9, the 80% level returns a parameter estimate of 0.612.

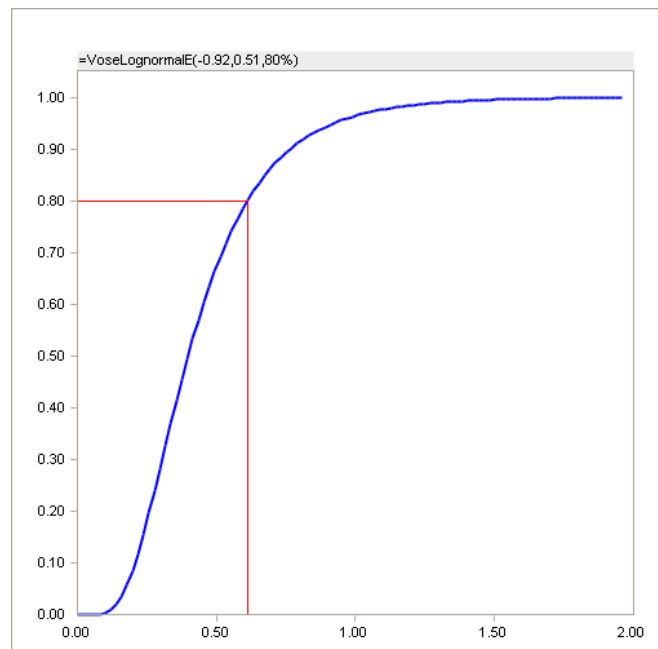


Figure 9 Inverting the Lognormal CDF to return a particular percentile

6 Quality Assessment

6.1 Data quality assessment

In their offer, the Project Team proposed to screen a selection of relevant input parameters (from different data sources) on their quality using the NUSAP approach. NUSAP (Numeral, Unit, Spread, Assessment, Pedigree) is a notational system which aims at better management and communication of uncertainty (Funtowicz and Ravetz, 1990) and has proven its value in other food safety quantitative microbial risk assessments (Boone et al., 2009b). The internal and external validity of the data was to be evaluated by the EFSA BIOHAZ WG members using four criteria specified in a pedigree: proxy, empirical, method and validation. The **proxy** criterion indicates how close or good a measure of the quantity which we model resembles the actual variable about which we want information. The **empirical** criterion evaluates the degree to which direct observations are used to estimate the parameter (field data versus indirect, modeled data or expert opinion). The **methodological rigor** refers to the norms used in collection, checking and validation of data and the degree of acceptance of these norms by the peer community of the relevant discipline. The **validation** criterion indicates the degree to which one was able to cross-check the data against independent sources.

The data quality was not assessed by the EFSA BIOHAZ WG members. Instead, they preferred to assess the quality of the input parameters for the different interventions that were obtained through a literature review. This assessment is described in section 8.1.

6.2 Assumptions assessment

6.2.1 Methodology

The NUSAP approach (see data quality assessment) was used to identify, prioritize and evaluate the key assumptions that were used in the model. The NUSAP/Pedigree method for the evaluation of assumptions has been successfully tested in environmental models (Kloprogge et al., 2005; van der Sluijs et al., 2005) and has been applied to QMRA by Boone et al. (2009a) to prioritize the key-assumptions in the Belgian QMRA model for *Salmonella* in the pork production chain, and to evaluate their subjective nature.

Each key assumption was assessed according to following criteria:

- Influence of situational limitations, reflecting to which extend the choice of the assumption was due to situational limitations, caused by data gaps, time limits, soft and hardware limitations or limited human resources;
- Plausibility, reflecting to which extend the assumption reflects the reality;
- Choice space, reflecting the number of alternative assumptions that could have been used;
- Agreement among peers, reflecting to which extend other experts would have used the same assumption;
- Influence on results, reflecting the impact of the choice of the assumption on the results, being the ability of the model to correctly predict the impact of the interventions on the human incidence for campylobacteriosis.

The pedigree matrix that was used for the assumption assessment is presented in Table II.

Table II Pedigree matrix clarifying the score for the 5 criteria for assumption assessment					
Criteria	Influence situational limitations	Plausibility	Choice space	Agreement among peers	Influence on results
Score					
4	Choice assumption hardly influenced	The assumption is very plausible (based on established theory, verified through peer review)	No alternatives are available	A large majority (90-100%) among peers would have made the same assumption	The assumption has little or no impact on the results
3	Limited influenced in choice assumption	Plausible (based on model with theoretical basis, empirically verified data)	Only 1 or 2 alternatives are available	Many experts (75%) would have made the same assumption	The assumption has only a local impact
2	Choice assumption moderately influenced	The assumption is acceptable (based on a simple model, extrapolated data)	Limited number of alternative are available	Several experts (50%) would have made the same assumption	The assumption greatly determines the results in a major step in the calculation
1	Important influence in choice assumption	Assumption is doubtful (based on not verified empirical data)	Wide range of alternatives are available	Few experts (25%) would have made the same assumption.	The assumption has a moderate impact on the end result
0	Totally different assumption had there not been limitations	The assumption is fictive or speculative	Any alternative is possible	Controversial assumption, hardly any expert (1%) would have made the same assumption	The assumption greatly determines the end result

Eight key assumptions (assumption 1 to 8 here below) were identified by the Project Team during a brainstorm session during the Project Team's second face-to-face meeting. Two more key assumptions (assumptions 9 and 10 here below) were defined by the EFSA BIOHAZ WG at the second Project Board meeting. Each assumption was presented to and discussed in detail by the EFSA BIOHAZ WG members at the second Project Board meeting to allow them to fully comprehend the context of the assumption. After that, each assumption was scored by all EFSA BIOHAZ WG members during the meeting, using a standard form.

The average score for all EFSA BIOHAZ WG members was calculated for each criterion and for each assumption. In addition, the Strength of the assumption, indicating its level of subjectivity, was calculated, being the average score for the four criteria: 1) Influence of situational limitations, 2) Plausibility, 3) Choice space, and 4) Agreement amongst peers. Also the standard deviation for the Strength and Influence on result was estimated to illustrate the scoring variability amongst the EFSA BIOHAZ WG members. The Strength and Influence on Result of each key assumption was plotted against each other in a diagnostic diagram to identify the assumptions with low overall pedigree strengths and strong estimated influence on the results as the weak links in the model.

6.2.2 Key assumptions

6.2.2.1 Assumption 1: The effects of the different interventions are independent of each other

The difference between independence and dependence of interventions is illustrated in Table III. In both cases, the initial prevalence is reduced when the interventions are applied. When interventions are independent the reduction in prevalence for respectively interventions A, B, and C (10%, 20%, and 30%) remains the same, indifferent from the other interventions that were applied. In case of dependence between interventions, synergism or antagonism between interventions may occur. In Table IIIb, the reduction by intervention B becomes 30% when it is preceded by intervention A.

Table III Illustration of the difference between independence and dependence of interventions

A. Interventions are independent				
Flock prevalence	80%	80%	80%	80%
Intervention A	72%	72%		72%
Intervention B	58%		64%	58%
Intervention C	40%	50%	45%	
B. Interventions are dependent				
Flock prevalence	80%	80%	80%	80%
Intervention A	72%	72%		72%
Intervention B	50%		64%	50%
Intervention C	35%	50%	45%	

6.2.2.2 Assumption 2: The effects of the interventions are always fractional

This assumption is illustrated by Table IV, which shows that the reduction imposed by an intervention is always proportional to the level of prevalence or concentration prior to intervention. This implies that implementing the interventions will never lead to zero or negative prevalence or concentrations (cfu/g).

Table IV Illustration of the difference between independence and dependence of interventions

A. Reduction in prevalence					
Initial prevalence		80%	60%	40%	20%
Intervention A	(-10%)	72%	54%	36%	18%
Intervention B	(-20%)	58%	43%	29%	14%
Intervention C	(-30%)	40%	30%	20%	10%
B. Reduction in log cfu/g					
Initial concentration		10	6	4	2
Intervention A	(-1 log cfu/g)	9	5	3	1
Intervention B	(-2 log cfu/g)	7	3	1	-1
Intervention C	(-3 log cfu/g)	4	0	-2	-4

6.2.2.3 Assumption 3: All the data used by the model are unbiased estimates

The uncertainty about the parameters that were used in the model is determined by the level of trueness (lack of systematic error or bias) and precision (lack of random error) (ISO, 1994) with which they were estimated from the target population.

Many sources of bias can cause a systematic error:

- Selection bias: not all sampling units have equal probability of being selected
- Non-response bias: required information cannot be obtained from all selected sampling units
- Response bias: incorrect measurement of information
- Operational bias: wrong implementation of sampling design
- Sample estimation bias: wrong calculation of estimates

When developing the model, it was assumed that all parameter estimates were unbiased, as no additional information on possible sources of bias was available. For example, the diagnostic test characteristics of the assays that were used for identifying the *Campylobacter* species and strains were not known. For the EFSA Baseline study, no information was available on the representativeness of the concentration measurements on the neck skin sample for the total *Campylobacter* load on the carcass.

As a consequence the uncertainty distributions that were fitted to the parameters only considered the sample size on which the estimations were based, being a measure of precision.

6.2.2.4 Assumption 4: Consumption of broiler meat originating from batches with negative test results will not lead to confirmed campylobacteriosis in humans

It is possible that the *Campylobacter* infection is introduced to a flock so close to the slaughter time that the pooled 10 ceaca samples in the EFSA Baseline study do not detect the infection. On the other hand, contamination on a carcass may be of such low concentration that it is below the detection level of the assays that were used. Therefore, carcasses from negative batches may nonetheless contain some *Campylobacter* through cross-contamination at the slaughter plant from other batches.

This may lead to a certain level of misclassification bias, which cannot be estimated and which we exclude by assuming that the loads which were not detected by testing ceaca or carcass samples are insufficient to incur infections in humans. This assumption is consistent with the CARMA project findings (Nauta et al., 2005a).

6.2.2.5 Assumption 5: The efficiency of immunity against exposure is constant in time

There is some evidence that *Campylobacter* infections induce short term immune responses in humans. This immunity may protect against clinical campylobacteriosis, depending on the time between a first and second exposure. We ignore this short term protection and assume that immunity is constant in time.

In practical terms this means that we are assuming that the reduction in prevalence or load of *Campylobacter* which would reduce the exposure to sub-infectious levels does not affect this short-term immunity. There are no data we know of that would allow us to build any other assumption.

6.2.2.6 Assumption 6: The levels of consumption of chicken are constant across the year

The prevalence of *Campylobacter* in flocks and on broiler carcasses shows a seasonal fluctuation. This fluctuation may have an impact on the seasonality of human campylobacteriosis but also on the efficacy of interventions under evaluation. The seasonality of human campylobacteriosis may also be influenced by a seasonal fluctuation of chicken meat consumption, which, in that case must be considered as a confounder. Little information on seasonal changes in chicken meat consumption is available. Therefore we have to assume that the level of consumption is constant throughout the year.

6.2.2.7 Assumption 7: The effectiveness of the interventions is equal in all countries

Information on the efficacy of interventions often results from experiments which do not consider geographical variation. For some interventions, specific efficacy information is available for some countries but not for all MS which are evaluated by the model. Given the lack of country specific information, it is assumed that the efficacy in terms of prevalence or concentration reduction is equal in all MS.

The impact of the intervention on the human incidence only differs on the level of application in the different MS. In applying interventions to each country in the model, the user must consider whether an intervention is practical and whether it should be applied only in certain periods of the year.

6.2.2.8 Assumption 8: All *Campylobacter* species and strains are equally susceptible to the different interventions

Different *Campylobacter* species or strains may cause different levels of campylobacteriosis incidence in humans and the prevalence of the different *Campylobacter* species or strains may differ across the MS. Data on the prevalence and concentration of different species and strains are incomplete in the EFSA baseline study data and the human incidence data. No solid information on the susceptibility of the different species, their load and pathogenicity is available.

In modelling the relationship between the *Campylobacter* concentration on broiler carcasses and the human campylobacteriosis incidence, and consequently assessing the effect of the different interventions, it has been necessary to assume that all *Campylobacter* species and strains are equally susceptible to the different interventions.

6.2.2.9 Assumption 9: The model accounts for all heterogeneity in the steps between the carcass concentration estimation and the exposure and all heterogeneity in the host-pathogen interaction.

Many events between the estimation of the *Campylobacter* concentration on the carcass after chilling and the exposure during consumptions of the boiler meat may have an influence on the risk for the human campylobacteriosis incidence. Some of these events are identified as interventions but others such as meat processing, preparation and consumption are difficult to assess. Moreover, the host-pathogen interaction

may differ, according to strains, consumer age, health status, etc., but again information on these interactions is not available.

The dose-response function model relates the *Campylobacter* concentration on broiler carcasses and the human campylobacteriosis incidence. Two different models were used with quite different mathematical bases. In principle, the dose-response function as applied within this model incorporates all heterogeneity in the steps between the carcass concentration estimation and the exposure and all heterogeneity in the host-pathogen interaction. However, the shape of this relationship in the real world is unknown. It is assumed that by using two quite different dose-response functions the impact of the uncertainty in this shape has been captured.

6.2.2.10 Assumption 10: The effect of interventions on caecal load is directly proportional on the load on the carcass after chilling

Some interventions, such as bacteriocins, can cause a reduction in *Campylobacter* concentrations in the caeca but not on the carcass after slaughter. Other interventions, such as freezing, will only have an impact on the *Campylobacter* concentration after slaughter but not in the caeca. The model uses *Campylobacter* concentrations that were estimated on carcasses after slaughter to assess the impact of interventions that reduce caecal load on farms. Therefore, a log-linear (i.e. proportional) relationship between caecal concentrations before slaughter and carcass concentrations after slaughter is assumed. As an alternative, the relationship could have been based on a mechanistic model, as proposed by Nauta et al. (2005a), but parameter estimates for this model are purely based on expert opinions rather than data, so it was considered more transparent to apply a simpler relationship.

6.2.3 Results

All assumptions were assessed for all criteria by 13 EFSA BIOHAZ WG members. Two members assessed assumption 2 double, separating between the pre-harvest (prevalence) and post-harvest (concentration) situation. The double assessments were considered as additional scorings when the results were analyzed.

The average scores for the NUSAP criteria and the overall strength of the 10 assumptions are presented in Table V. Figure 1 shows the diagnostic diagram where the overall strength of the different assumptions is plotted against their expected influence on the model result, being the expected change is the incidence of reported human campylobacteriosis induced by applying the interventions under evaluation. The diagnostic diagram shows that assumptions 1, 3, 9 and 10 are highly subjective and tend to have a high influence on the model result.

6.3 Output validation

The mathematics have been validated by testing that applying and removing interventions decreases and increases the mean log load and usually the estimated human health impact respectively. This shows that each intervention appears in the code and the logic gives the correction intuitive direction of influence. The plausibility of the results of the scenario analyses (Section 7) have been commented and validated by the Project Team experts for their individual countries.

The EFSA BIOHAZ WG members will also need to consider independently whether the output values look 'realistic'.

Assumption	Influence of situational limitations	Plausibility	Choice space	Agreement amongst peers	Strength	Influence on result
1	1,2	1,3	2,1	3,1	1,9	1,4
2	2,1	2,1	2,6	2,7	2,4	1,7
3	1,2	1,2	1,7	2,1	1,6	1,5
4	2,6	2,4	2,1	2,2	2,3	2,7
5	1,9	2,1	2,6	2,8	2,3	2,4
6	2,4	1,9	2,2	2,7	2,3	2,5
7	1,5	1,7	2,4	2,8	2,1	1,9
8	1,8	1,5	2,6	2,3	2,1	2,1
9	1,4	1,3	2,2	1,8	1,6	1,6
10	1,5	1,9	2,0	1,8	1,8	1,5

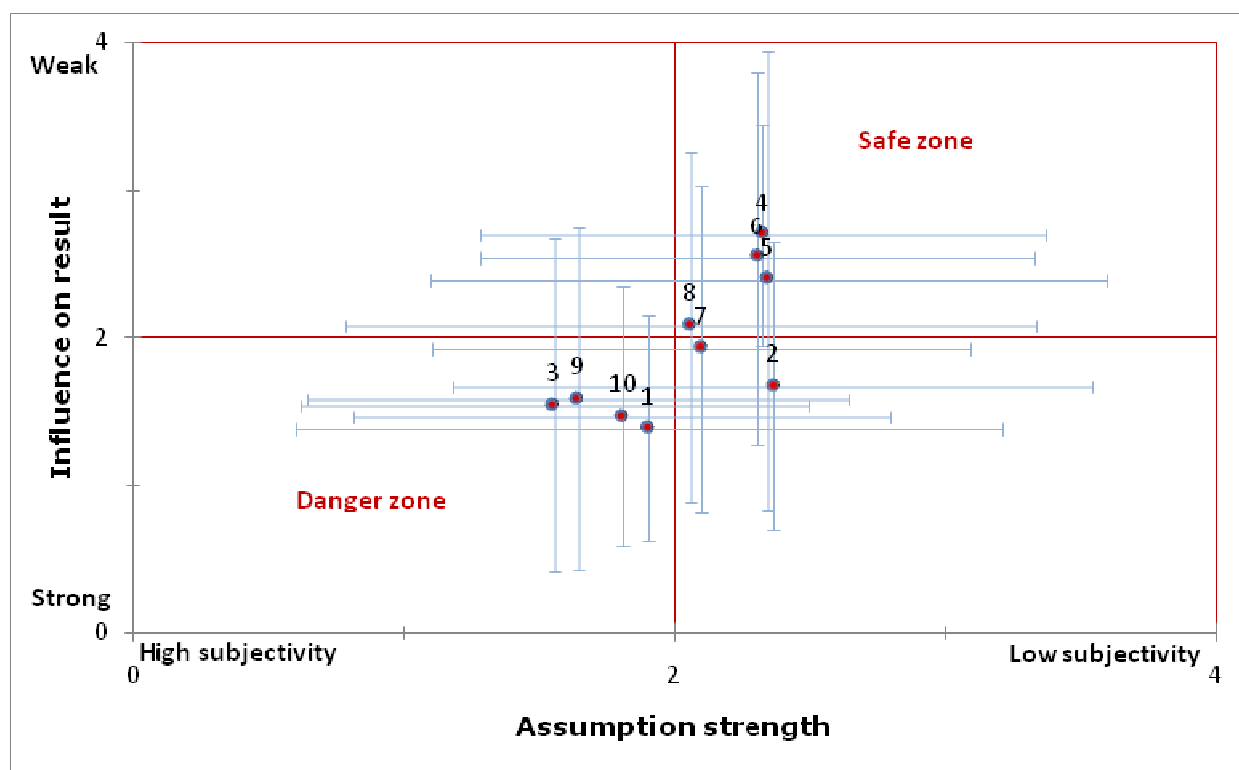


Figure 10 Diagnostic diagram showing the relationship between the scores for the strength of the key assumptions and their influence on the result (with standard deviation).

6.4 Sensitivity analysis

6.4.1 Sensitivity to the form of the dose-response model used

The form of the dose-response function remains an assumption forced by the limitations of the available data. However, the model estimates the dose-response parameters using estimated true (i.e. adjusted for under-reporting and attribution to chicken meat) number of human cases, and uses the precise same dose response function to estimate the new level of true number of human cases one might see when changes to the interventions are made. This limits the sensitivity to the form of the dose-response function. The model ultimately calculates the fractional change in expected recorded cases induced by the change in interventions as:

$$\text{Fractional change} = 1 - \frac{\text{New Level}}{\text{Old Level}} \quad \text{Equation 5}$$

An analysis was performed to demonstrate the degree of sensitivity of the model's output to the selection of dose-response function, using the following scenario:

Country: Denmark

Change in interventions applied in future state:

1. Bacteriophages (from not applied to 25%/30%/40%) in indoor farms only using the Wagenaar reference giving a 1 log reduction in load
2. Fly screens (from not applied to 50%/60%/80%) of indoor farms only between June and October

Indoor farms represent 99.5% of all Danish poultry farms. The simulation results incorporating statistical uncertainty of input parameters are as follows:

Simple exponential dose-response model

- Mean fractional reduction in human campylobacteriosis: 40.06%
- Standard deviation: 3.88%

Beta-Poisson dose-response model

- Mean fractional reduction in human campylobacteriosis: 39.41%
- Standard deviation: 3.91%

Figure 11 plots the two cumulative distributions of fractional reduction in human campylobacteriosis using each of the two dose-response functions, together with the Combined dose-response model weighting each of the two models equally. The two plotted lines for the Simple Exponential and Beta-Poisson models are in very close agreement, as are the summary statistics above, demonstrating that the model output for this scenario is fairly insensitive to the choice of dose-response function. In general, similar results have been observed in other scenarios we have tested. The user is recommended to look at both results in assessing interventions.

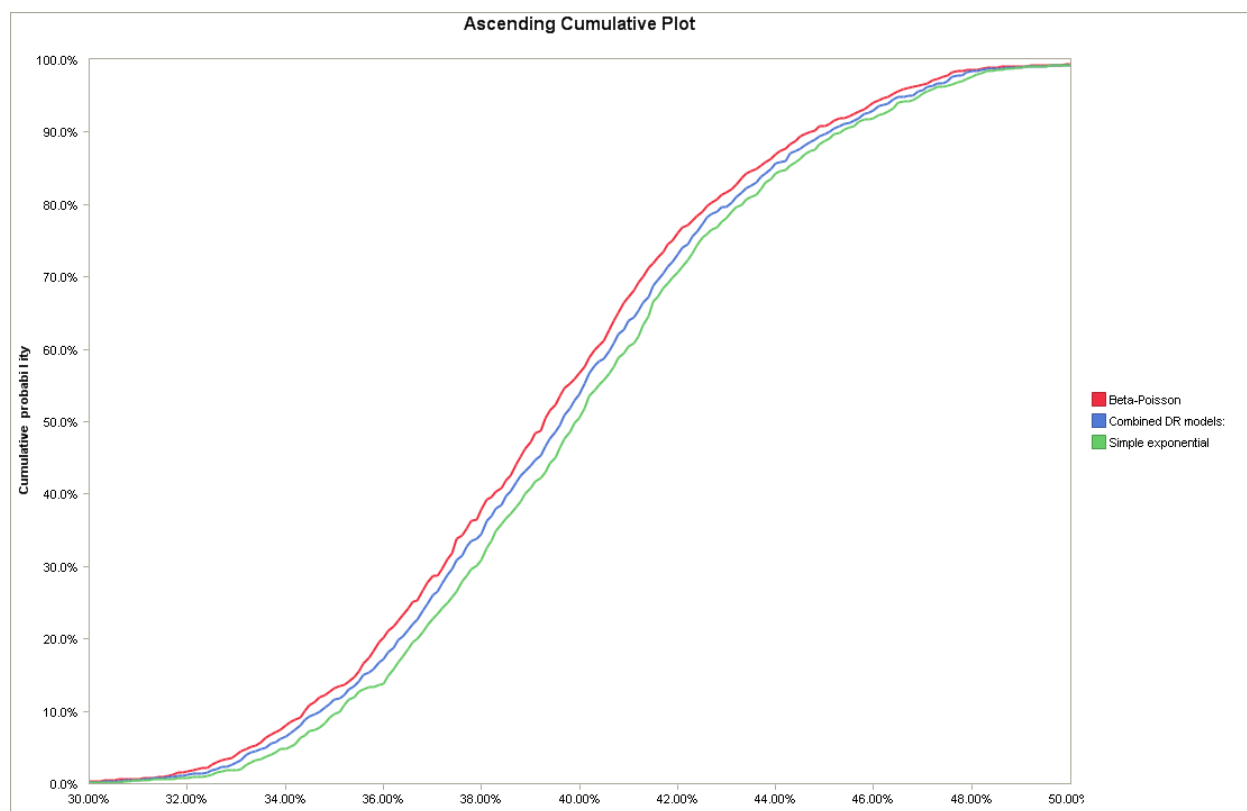


Figure 11 Comparison of cumulative distributions for the two dose-response functions using the Danish scenario described above, together with the Combined model.

6.4.2 Model parameter uncertainty

In stochastic mode the model automatically stores the change in between flock prevalence, within-flock prevalence and log mean load for any intervention that has been altered between current and future states, as well as other stochastic control parameters. These values are used within ModelRisk to produce a variety of sensitivity analysis reports. The most common sensitivity analysis is a *Tornado chart*, an example of which is shown in Figure 12. Tornado charts can be plotted after a simulation run from within the ModelRisk Results Viewer window by clicking the following button on the ribbon (shown with a green box):



The vertical axis lists the variables with a statistically significant rank order correlation to the selected model output. The level of correlation is shown on the x-axis scale. One should focus on the absolute size of correlation, not the sign (negative or positive) which indicates the direction of influence. Changes in prevalence and mean log load have been defined for the purposes of sensitivity analysis so that they should show a positive correlation with the fractional reduction in human health cases. This makes reviewing the tornado chart easier and allows a quick check that correlations are in the expected direction.

In the example of Figure 12 the uncertainty in the estimated reduction in human campylobacteriosis using the Combined model is most sensitive to the uncertainty in the effect of fly-screens. After that, the result

has some small sensitivity to the selection of model (Simple Exponential or Beta-Poisson) and almost no sensitivity to the uncertainty in the effect of bacteriophage.

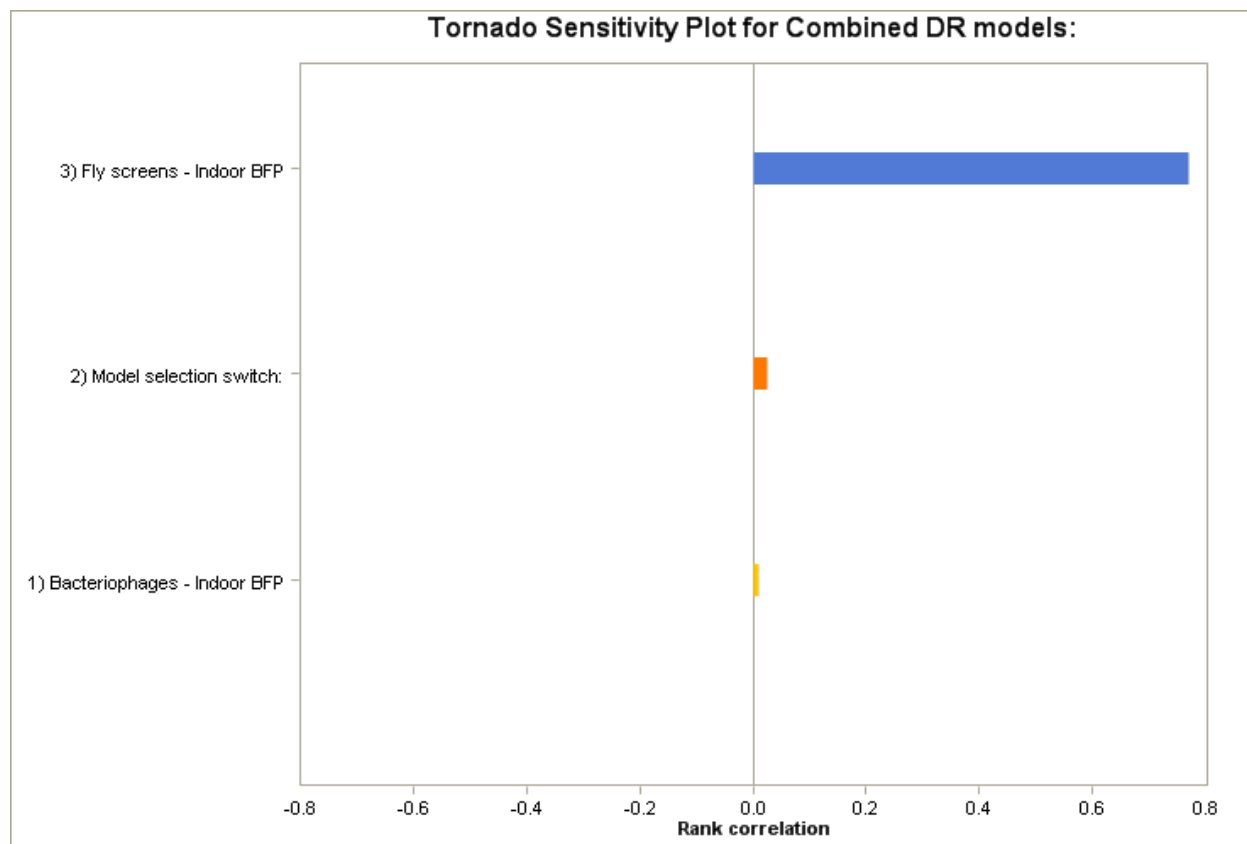


Figure 12 Example Tornado Chart

Other graphical displays of correlation are also possible. Various spider plots give a more quantitative explanation of the degree of influence on the output, and scatter plots show the pattern of the relationship between each input and output. These graphs are explained in detail in the ModelRisk help file.

7 Scenario analysis

The model predicts the reduction of human campylobacteriosis resulting from interventions made at different stages of the food chain. Therefore, it is able to evaluate different scenarios where either:

1. the reduction in human campylobacteriosis caused by the implementation of a subset of interventions, or;
2. the required performance of a subset of interventions to obtain a target level of human campylobacteriosis reduction is assessed.

Many scenarios can be identified and this choice is open to the model user. We have worked together with EFSA staff in evaluating various scenarios defined by the WG. The results are provided in a separate report by EFSA to the WG.

7.1 Selection of interventions

The selection of interventions to be included in the model occurred in three stages. First, the member of the EFSA BIOHAZ WG prioritized all possible interventions according to their capability of reducing the *Campylobacter* prevalence or concentration in the food chain. Table VI presents the short list of interventions that were scored according to their priority to be included in the model. All of these interventions were included in the model.

In a second stage, a literature review was performed by the Project Team seeking for experimental or observational studies containing information on the level of *Campylobacter* prevalence or concentration reduction resulting from implementing each of the short-listed interventions. The publications that have been consulted for the literature review are provided in Table VII. For each intervention and each study following information was obtained, when available:

- The number of study units in the treatment and control group;
- The number of positive flocks or batches in the treatment and control group or before and after treatment (flock/batch prevalence);
- The number of positive birds within a flock or batches in the treatment and control group or before and after treatment (within flock/batch prevalence);
- The mean concentration reduction in caeca or carcass sample on a Log10 scale (e.g., Log cfu/g, Log cfu/ml, Log cfu/carcass, etc);
- The standard deviation of the concentration reduction in caeca or carcass sample on a Log10 scale (e.g., Log cfu/g, Log cfu/ml, Log cfu/carcass, etc).

In a third stage, the quality of the input data on the interventions efficacy was assessed by evaluation the quality of the studies they originated from. Again the NUSAP quality assessment procedure (see Section 6) was used.

The different studies were evaluated using following NUSAP criteria:

- **Proxy:** how close is the measured quantity to the information we seek
- **Empirical basis:** degree to which direct observations, measurements and statistics are used to estimate the parameter
- **Method:** norms for methodological rigor in this process applied by peers in relevant disciplines
- **Validation:** degree to cross-check data and assumptions used to produce the numeral of the parameter against independent sources.

Table VI Interventions included in the model and their priority as assessed by the EFSA BIOHAZ WG

		Priority for QMRA
FARM		
Hygiene/biosecurity		14
Bacteriophages/bacteriocins		8
Vaccination		8
Fly screens		5
Thinning		5
Treatment of water with organic acids		3
Slaughter age		2
TRANSPORT/LAIRAGE		
Control transport/lairage time		3
Crates/modules/vans treatment		1
SLAUGHTER		
Detection/re-processing of highly fecal-contaminated carcasses		9
Prevention of fecal leakage / cloacal plugging		3
Physical decontamination		
	Freezing	7
	Steam-ultrasound	6
	Heat treatment (RTE-meals)	4
	Crust-freezing	3
	Steam or hot water	3
	Irradiation	2
Chemical decontamination		
	Organic acids; acetic-, lactic-	9
	Acidified electrolyzed oxidizing water	4
	Acidified sodium chlorite (ACS)	4
	Chlorine	3
	Trisodium phosphate	1
Logistic slaughter (the slaughter of negative flocks before the positive)		1
Scheduled slaughter (scheduling positive flocks for special treatment) [interventions only applied after slaughtering of flocks that were tested positive at some stage before slaughtering. Time lags may vary between one week and a few hours depending on analytical methods and logistic requirements]		9

All studies were listed in a web-application that could be consulted by the EFSA BIOHAZ WG members for the quality assessment. In addition the EFSA BIOHAZ WG members were asked to provide their expertise on the specific intervention.

The quality assessment was performed by on average 3 WG experts, which was not sufficient to include the results of the quality assessment in the model. Therefore, the quality of the different intervention

inputs were not taken into account when obtaining the results for the scenarios described here-after. But, the functionality for considering the quality remains available in the model.

Table VII Publications that have been consulted by the Project Team to obtain input on the efficacy of the interventions that have been proposed by the EFSA BIOHAZ WG. The parameters (concentration, between-flock prevalence (BFP) and within-flock prevalence (WFP)) for which information is available are also indicated.

Intervention	Information	Reference
FARM		
Hygiene/biosecurity	BFP	(Katsma et al., 2005) Gibbens et al., 2001)
Bacteriophages	concentration	(Wagenaar et al., 2005; El-Shibiny et al., 2009b)
Bacteriocins	concentration	(Stern et al., 2005; Stern et al., 2006; Line et al., 2008)
Vaccination	concentration	(Wyszynska et al., 2004; de Zoete et al., 2007; Buckley et al., 2009)
Fly screens	BFP	(Hald et al., 2007)
Treatment of water with organic acids	concentration	(Chaveerach et al., 2004)
Thinning	BFP	Estimated from EFSA Baseline Study data (except Greece)
Slaughter age	BFP	Estimated from EFSA Baseline Study data (except Greece)
TRANSPORT/LAIRAGE		
Control transport/lairage time	concentration	(Berrang et al., 2004)
Crates/modules/vans treatment	concentration	(Allen et al., 2008)
SLAUGHTER		
Detection/re-processing of highly faecal-contaminated carcasses	concentration	(Kemp et al., 2001)
Prevention of faecal leakage /cloacal plugging	concentration WFP	(Musgrove et al., 1997; Berrang et al., 2001; Buhr et al., 2003)
Physical decontamination	concentration	(Bhaduri and Cottrell, 2004; Purnell et al., 2004; Nauta et al., 2005b; Birk et al., 2006; Georgsson et al., 2006; Sandberg et al., 2006; Whyte et al., 2006; Corry et al., 2007; James et al., 2007; Boysen and Rosenquist, 2009; El-Shibiny et al., 2009a)
Chemical decontamination	concentration	(Hwang and Beuchat, 1995; Kemp et al., 2001; Yang et al., 2001; Park et al., 2002; Warf and Kemp, 2002; Oyarzabal et al., 2004; Hinton and Ingram, 2005; Kim et al., 2005; Zhao and Doyle, 2006; Northcutt et al., 2007; Sexton et al., 2007; Loretz et al., 2009; Riedel et al., 2009)
Logistic slaughter	BFP	Personal communication with Merete Hofshagen
Scheduled slaughter	BFP	Personal communication with Merete Hofshagen

8 General discussion

8.1 Data quality, gaps and requirements

One of the values of the Model is that it only uses empirical data to assess the relationship between *Campylobacter* prevalence and contamination level and reported human campylobacteriosis cases. *Campylobacter* prevalence and contamination level are obtained from the EFSA baseline survey and the human campylobacteriosis cases are reported in the Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union.

The EFSA baseline survey was organized in such way that it would comply as much as possible to the principle of 'equal probability sampling', i.e. each unit in the population has equal probability to be sampled in the survey. Equal probability sampling contributes to obtaining 'unbiased estimates'. Still, the EFSA baseline survey data may incorporate an important level of reporting bias. For example, each skin sample that was taken after chilling was examined using a detection and enumeration methods. A number of samples that tested negative for the detection method tested positive for the enumeration method and vice versa. No diagnostic test characteristics were available for the methods that were used. Therefore the transition from apparent to true prevalence was not possible. In order to minimize the reporting bias, the results from the detection and enumeration method was interpreted in parallel where the sample was considered as positive if for at least one of the two test a positive test result was obtained.

The human campylobacteriosis cases that are reported in the Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union are prone to under-reporting bias. The collection of these data is not harmonized between MS and the number of reported cases highly depends on the surveillance systems that are in place in the different MS. The under-reporting is not really a problem when the model is used for individual countries as it estimates the fractional reduction of human campylobacteriosis cases. Different levels of under-reporting in different MS make a comparison of the efficacy of certain interventions between different MS impossible? To solve this problem, the EFSA BIOHAZ WG suggested using a under-reporting ratio to bring the reported cases to the same magnitude for all MS.

Input on the efficacy of the interventions that were evaluated by the model, was obtained through a literature review carried out by the Project Team. The list of studies that were consulted was not exhaustive, most of the studies were carried out in experimental setting with low numbers of experimental units and the reported efficacy of some interventions varied considerably between different studies. Since we were not able to differentiate the quality of different studies, we considered all efficacy values with equal weight in the model when analysis the scenarios stated in this report. The input values on the efficacy of the interventions can certainly be improved by the experts of the EFSA BIOHAZ WG.

In addition to their efficacy the Model also uses the level of application of the different interventions in the different MS. This information was obtained by questionnaire for the different MS that were represented by the Project Team (BE, DK, GR, NO, PT). These data are not yet available for the remaining MS.

Campylobacter prevalence and contamination level data for most MS are available from the EFSA baseline survey. The human campylobacteriosis cases for most MS are reported in the Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union. Following additional data are required when EFSA wishes to use the model for risk assessment in all MS:

1. The minimum, most likely and maximum level of application of the different interventions in the different MS need to be obtained for broiler batches reared in both 'indoor' and 'outdoor' farms.
2. The input data on the efficacy of the different interventions can be improved by the experts if the EFSA BIOHAZ WG.

8.2 Values and limitations of the model

The focus of building the model has been to base its structure on the decision questions posed by the WG and the data that were available to support the structure. We have avoided making any indefensible assumptions and believe that the resultant model provides the most comprehensive support to the WG possible.

The model needed to be applicable to any MS for which data are available. Most importantly, in contrast with other farm-to-fork risk assessment model, the model does not explicitly incorporate a section dealing with handling, storage and cooking of chicken products post-chilling because this is a highly complex system for which no reliable models are available with solid data for estimating the parameters, particularly when considering the variations in behavior that are likely between MS.

The resultant model is what is commonly termed a two-dimensional risk assessment model, in that it keeps separate probability estimates and statistical uncertainty about the parameters. Probability estimates are calculated directly whilst statistical uncertainty is represented through Monte Carlo simulation. This enables the user to evaluate the vulnerability of the model outputs to the statistical uncertainty in the input parameters due to the amount of data available.

The model can run in *deterministic* or *stochastic* mode. In *deterministic* mode, the model uses single point estimates of uncertain parameters and therefore returns single values for each model output. One immediately gets a reasonable value for the estimate in reduction of human cases in a single recalculation of the model. This can be very helpful for quickly comparing many scenarios.

Stochastic mode is designed to give a better estimate for the scenarios selected by the WG because it incorporates the statistical uncertainty of the model outputs. The WG might, for example, find a particular set of interventions to be appealing in deterministic mode, but then find that the statistical uncertainty is too great to be able to provide a recommend that set of interventions.

A limitation of the probability domain of the model being performed with calculations, rather than simulation, is that one cannot directly incorporate sub-models of systems that involve feedback loops or sequential dependence. For example, it is not possible to look at the effect of cross-contamination at the slaughter plant for a *Campylobacter*-free flock that is preceded by an infected flock. Technically, it is possible to extend the model to take these effects into account through VBA functions that internally simulate such a system and then return the probability parameters as outputs. We investigated this possibility but excluded such sub-routines because there were no reliable parameter estimates for such systems and, even if there were, it would slow down the model performance to a prohibitive extent.

9 Getting started with the model

9.1 Running scenarios

9.1.1 Running a single scenario

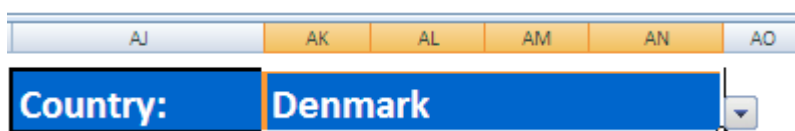
The model requires that one has Microsoft Excel with the ModelRisk 3.0 add-in from Vose Software (www.vosesoftware.com) installed and running and that the Excel security settings allow VBA macros to run. Clicking the ModelRisk icon on the desktop, or selecting Start|All Programs|Vose software|ModelRisk|ModelRisk will open both Excel and ModelRisk. This should be done first before loading the model file. When loading the model into Excel, the user may need to do a global spreadsheet recalculation (CTRL+ALT+F9) to get Excel to recognize all of the VBA code and add-in functions.

The model can operate in two modes: *Deterministic*; and *Stochastic*. The user can switch between these modes by changing the setting in worksheet 'Inputs & Controls'. In *Deterministic* mode the model uses the best available single point estimates for the model's parameter values. This means that the resultant calculation of the fractional change in human illness rates is also a single point estimate. This is the standard mode of use of the model because it provides quick estimates of the output, allowing the user to explore different combinations of interventions in a consistent framework.

The *Stochastic* mode is used to perform a statistical sensitivity analysis of the effect of a set of interventions. It requires running a simulation with some 500 samplings to get reasonable results. It is recommended that this is performed once the user has decided on a set of interventions. In *Stochastic* mode, the model simulates the statistical uncertainty of input parameters according to the amount and quality of data used to estimate the parameters. Annex 1 provides the formulae used for the parameters in both settings.

The user should begin by going through the sheets with blue tabs in order to select the references that they feel are most robust in estimating the effect of interventions. Section 9.2 explains how to select or change the reference inputs. This will probably only need to be done with the first use of the model. Clicking Excel's save will save the user's preferences for references to be used.

The easiest way to run a single scenario for a certain country is to go into the 'Country' sheet, select the country of interest from the drop down menu (cell AK2) :



Then the user enters the application fractions of the interventions for the future state (cells BU20-BZ116)(Figure 13).

	A	B	C	BU	BV	BW	BX	BY	BZ
1									
2	6	Country: Denmark		Cells marked with a red background can be changed by the user					
3									
4									
5									
6									
7		Number of broilers slaughtered		Reset inputs to default values					
8		Number of reported human cases							
9		Number of actual attributable cases							
10		Expected infections per broiler							
11									
12		Indoor		Indoor	Outdoor				
13				Future State Fractions					
14		Intervention							
15									
16		Starting point		Min	Mode	Max	Min	Mode	Max
17									
18									
19		Hygiene/biosecurity		62%	75%	94%	0%	0%	0%
20									
21		Contaminant/prov							
22		Bacteriophages		25%	30%	40%	0%	0%	0%
23									
24		Contaminant/prov							
25		Bacteriocins		0%	0%	0%	0%	0%	0%
26									
27		Contaminant/prov							
28		Vaccination		0%	0%	0%	0%	0%	0%
29									
30		Contaminant/prov							
31		Fly screens		50%	60%	80%	0%	0%	0%
32									
33		Contaminant/prov							
34									
35		Thinning	Proportion						
36									
37		Contaminant/prov							
38		Water treatment with organic acids		0%	0%	0%	0%	0%	0%
39									
40		Contaminant/prov							
41		Slaughter age	Current state						
42									
43		Contaminant/prov							
44		Control transport/lairage time		0%	0%	0%	0%	0%	0%
45									
46		Contaminant/prov							
47		Crates/modules/raas treatment		0%	0%	0%	0%	0%	0%
48									
49									
50		Slaughter plant							
51									
52		Logistic slaughter		0%	0%	0%	0%	0%	0%
53									
54		Contaminant/prov							
55		Scheduled slaughter		0%	0%	0%	0%	0%	0%
56									
57		Contaminant/prov							
58		Detection of highly faecal-contaminated		20%	50%	80%	20%	50%	80%
59									
60		Contaminant/prov							
61		Prevention of faecal leakage		0%	0%	0%	0%	0%	0%
62									
63		Contaminant/prov							
64		Cross-contamination	Variance reduction						
65									
66									

Figure 13 Selection of country and application fractions for future state scenarios

Note that, by clicking the ‘Hide all calculations’ button, the user can hide the columns and rows performing calculations leaving only the areas that require user input, which makes the model easier to use. Once clicked, the button changes to ‘Show all calculations’: clicking this will reveal all calculations so the user can follow the mathematics involved.

By default, the future state application fractions are set to the current state fractions. To see what the effect is of applying one or more interventions in the future state that are not applied in the current state, the user just has to enter the desired fractions of application for those interventions. The user can set all values back to the default state by clicking the ‘Reset inputs to default values’ button shown in the above Figure. If the model is in ‘Deterministic’ mode, one can just read off the single value outputs (fractional reduction in level of illness, reduction in log10 mean load, etc) from the Model Outputs section under ‘Deterministic outputs’, as shown in Figure 14.

	CA	CB	CC	CD	CE	CF	CG	CH
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43								
44								

Caution: the model has back-calculated a between flock prevalence > 1 at row 124

Model Outputs

Total cases current state: 3,475

Total cases future state: 2,028

Deterministic outputs:

Fractional reduction in human cases (simple exponential): 41.63%

Indoor only 41.90%

Outdoor only 0.00%

Fractional reduction in human cases (Beta-Poisson): 40.84%

Reduction in log10 mean load indoor / outdoor: 0.34 0.00

Fractional reduction in BFP indoor / outdoor: 36.96% 0.00%

Fractional reduction in WFP indoor / outdoor: 0.00% 0.00%

Combined DR model

Model selection switch: 2

Combined DR models: 40.84%

Stochastic outputs

Fractional reduction in human cases (Simple exponential)

Mean: 40.06%

St Dev: 3.88%

5th perc: 34.02%

50th perc: 39.92%

95th perc: 46.92%

Figure 14 Model Outputs section. Note a panel above the results table indicates if there are any potential problems that the user may wish to consider.

If the model is set to ‘Stochastic’ mode, one needs to specify the number of samples to run in ModelRisk and click the Start simulation button, as shown in Figure 15.

This will make ModelRisk begin simulating. The model is set up to automatically collect the input data to determine the statistical uncertainty around the fractional change in human illness rates and produce sensitivity analyses.

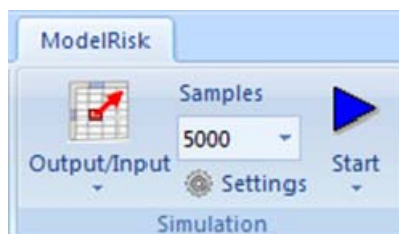


Figure 15 Running a 5000 samples iteration in ModelRisk

Once the simulation has finished, the statistics of the outputs will appear in the Model Outputs section under ‘Stochastic outputs’. The ModelRisk Results window will also appear. The user is then able to create any graphs required, and to save the entire report as a file that can be opened at any time or sent to colleagues who can view the report and make other graphs (density and cumulative plots, scatter plots, various tornado and spider sensitivity plots) using the free ModelRisk Results Viewer application, which can be downloaded from www.vosesoftware.com/resultsviewer.php

9.1.2 Running multiple scenarios

To run multiple scenarios in one go, one can use the ‘Scenarios’ sheet in the model. This sheet allows the user to enter up to 15 different scenarios, regardless of the country in which the future scenarios are applied. Figure 16 shows the layout of this sheet.

A B C D E F G H I J K L M N O P Q R S T U V W X Y Z																											
1	Country: Denmark										Run Scenarios																
2																											
3																											
4																											
5	Current state Denmark										Future state scenarios																
6																											
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Figure 16 Sheet ‘Scenario’ used for running multiple scenarios

For every scenario that needs to be run, the user has to enter the following information:

- Country (on line 7)
- Yes/No switch indicating if this scenario should be included in the simulation (on line 10)
- Application fractions

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
7	Name	Weight	Mean	StDev	Skewness	Kurtosis	n											Reference
8				(optional)	(optional)	(optional)												
9	Stern 2005	1	5.5	0.6			10											Stern 2005
10	Stern 2006	1	7.03	0.75														Stern 2006
11	Line	1	3.96	0.8			10											Line et al 2008 table 6
12		1																
13		1																
14		1																
15		1																
16	Expert opinion	1	8.333333	0.388889	0.305441	2.4												
17			Min	Mode	Max													
18	Expert opinion input		7	8	10													

Figure 18 Data and descriptions from references

As shown in Figure 18, a reference must always have a value for the mean log-reduction, and can optionally have information on the standard deviation, skewness, kurtosis and the number of observations on which the efficacy was estimated. Standard deviation, rather than variance which is used in the model, is provided as an input value because this is the more usual statistics reported in papers. There is space to enter 7 references, plus an ‘expert opinion’ reference. For the expert opinion, min, mode and max are entered on line 18 and the moments of a triangular distribution based on these inputs are then automatically calculated on row 16. The weights for all references are set to ‘1’ by default, but can be changed to assign more weight to one or more particular references.

The switches in columns B and D allow the user to select which reference should be used in ‘Deterministic’ mode and which references to use in ‘Stochastic’ mode. Since in ‘Deterministic’ mode the model returns one single output value, it means that it is based on one input reference per intervention. This can be either one of the 7 references or the ‘expert opinion’ line. When more than one reference is selected in this mode, an error message will pop up saying only one reference can be selected. In ‘Stochastic’ mode however, more than one reference can be selected to use in the model. When running a Monte Carlo simulation, the model will pick in every sample at random one of the selected references. References will be more likely to be picked during the simulation if they have a higher weight than other references.

The section between line 20 and 26 is only for calculation purposes, and picks the right central moments to put in the model depending on the model mode (deterministic or stochastic) and on the selected inputs. The addition of uncertainty around the central moments in ‘Stochastic’ mode is described in paragraph G of the Annex.

9.2.1.2 Adding/removing references

The way the intervention input sheets are constructed makes it very easy to add or remove references. The user can just delete the information (name, weight, mean ..., notes) from a line and enter new information or leave it blank. It is possible for the expert opinion line to enter the central moments directly on line 16, although this is not recommended as it is much less intuitive to have an expert enter the mean, standard deviation, skewness and kurtosis rather than the min, mode and max for the effect of an intervention. So it is best to enter these expert opinion inputs on line 18 as shown in Figure 18.

9.2.1.3 Inputs for BFP/WFP reduction

The inputs for the between flock prevalence (BFP) and the within flock prevalence (WFP) are similar to the load reduction inputs, except that the inputs are now the number of positive cases out of a total number tested, before (control) and after (treatment) applying an intervention (Figure 19). This results in values for the prevalence before and after applying the intervention, which are then used to calculate the

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	
1															
2	Control parameters														
3															
4	Model mode:							1 Stochastic							
5															
6	Include weightings of references?							0 No							
7															
8	Conversion from grams skin to carcass:							100 g skin/carcass							
9															
10	Cross-contamination: absolute reduction in variance:							Current		Future					
11								2		2					
12															
	Dose-Response model weights:							Simple exponential		1		Beta-Poisson		1	

Figure 20 Control parameters on sheet 'Inputs & Control'

9.2.2.2 Input data

The rest of the 'Inputs & Control' sheet contains data for every MS that is then used in the model. Here is an overview of the data located in this sheet:

- Current intervention application fractions
- Number of broilers slaughtered
- Array related to the reported human cases including:
 - Number of reported human cases of campylobacteriosis
 - Reporting factor based on a reporting table
 - Attribution factor
- Fraction of indoor and outdoor farms
- Results of the logistic regression performed on slaughter age and thinning data from the EFSA baseline study
- Log10 cfu load and prevalence at slaughter plant from the EFSA baseline study

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Annex

Annex 1: Mathematical formulae used in the model

A. Moment conversions

Almost any distribution can be characterized by its moments. Typically, two types of moments are distinguished: raw (or uncorrected) moments and central (or mean-centered) moments. Because it is easier to perform calculations on the raw moments, but the central moments are more intuitive (they are very closely related to the Mean, Variance, Skewness and Kurtosis of a distribution as will be shown below), it is important to be able to convert a set of raw moments to central moments and vice versa.

1. Raw to central

The conversion from a set of raw moments of a distribution to the central moments of that distribution is based on the following four equations:

$$\mu_1 = \mu'_1$$

$$\mu_2 = \mu'_2 - \mu^2$$

$$\mu_3 = \mu'_3 - 3\mu'_2\mu + 2\mu^3$$

$$\mu_4 = \mu'_4 - 4\mu'_3\mu + 6\mu'_2\mu^2 - 3\mu^4$$

where μ_x are the central moments and μ'_x are the raw moments.

The Mean, Variance, Skewness and Kurtosis of a distribution are defined as:

$$\text{Mean} = \mu$$

$$\text{Variance} = \mu_2$$

$$\text{Skewness} = \frac{\mu_3}{(\text{Variance})^{3/2}}$$

$$\text{Kurtosis} = \frac{\mu_4}{(\text{Variance})^2}$$

2. Central to raw

If we inverse the previous formulae, we get the conversion from central moments to raw moments:

$$\mu'_1 = \mu_1$$

$$\mu'_2 = \mu_2 + \mu^2$$

$$\mu'_3 = \mu_3 + 3\mu_2\mu + \mu^3$$

$$\mu'_4 = \mu_4 + 4\mu_3\mu + 6\mu_2\mu^2 + \mu^4$$

B. Moment calculations for combinations of two variables

1. $A + B$

If we want to add up two variables A and B , both defined by their four moments, we have to perform the calculations on the four raw moments of the variables. Let $\mu'_{k,A} = E[A^k]$ with $k = 1, 2, 3, 4$ be the raw moments of variable A and let $\mu'_{k,B} = E[B^k]$ with $k = 1, 2, 3, 4$ be the raw moments of variable B , then the raw moments for variable $Z = A + B$ are equal to:

$$E[Z] = E[A] + E[B]$$

$$E[Z^2] = E[A^2] + 2 * E[A] * E[B] + E[B^2]$$

$$E[Z^3] = E[A^3] + 3 * E[A^2] * E[B] + 3 * E[A] * E[B^2] + E[B^3]$$

$$E[Z^4] = E[A^4] + 4 * E[A^3] * E[B] + 6 * E[A^2] * E[B^2] + 4 * E[A] * E[B^3] + E[B^4]$$

For example, we might have data on the moments for the log10 load post-chilling A and have data on the effect of bathing the carcasses in acid afterwards B . If acid baths reduce the log10 load, the mean of B will be negative. If acid bathing occurs immediately after chilling, then the log10 load post acid bathing Z is then $A+B$.

2. $A - B$

A similar set of formulas are used to calculate the resulting raw moments of the difference of two variables $Z = A - B$:

$$E[Z] = E[A] - E[B]$$

$$E[Z^2] = E[A^2] - 2 * E[A] * E[B] + E[B^2]$$

$$E[Z^3] = E[A^3] - 3 * E[A^2] * E[B] + 3 * E[A] * E[B^2] - E[B^3]$$

$$E[Z^4] = E[A^4] - 4 * E[A^3] * E[B] + 6 * E[A^2] * E[B^2] - 4 * E[A] * E[B^3] + E[B^4]$$

For example, we might have data on the moments for the log10 load post-chilling A and have data on the effect of chilling the carcasses B . If chilling reduces the log10 load, the mean of B will be negative. The log10 load *prior* to chilling Z is then $A-B$. In other words, we are able to work backwards from an observed level of contamination through what the contamination levels must have been prior to various stages in processing that have already occurred. This is possible using moment calculations, but would not be possible using more conventional Monte Carlo simulation.

3. $A + p*B$

Consider the situation in which only a fraction p of flocks apply some intervention. This means that a random sample of flocks will have probability p of sampling a flock with some intervention. If the flock starts with log load distribution A and the intervention adds a log load distribution B to arrive at a log load distribution Z then we have::

$$E[Z] = E[A] + p * E[B]$$

$$E[Z^2] = E[A^2] + 2 * p * E[A] * E[B] + p * E[B^2]$$

$$E[Z^3] = E[A^3] + 3 * p * E[A^2] * E[B] + 3 * p * E[A] * E[B^2] + p * E[B^3]$$

$$E[Z^4] = E[A^4] + 4 * p * E[A^3] * E[B] + 6 * p * E[A^2] * E[B^2] + 4 * p * E[A] * E[B^3] + p * E[B^4]$$

For example, the formula for $E[Z^2]$ is derived as follows. Z is the composite of taking a value from A with probability $(1-p)$ and a value from $A+B$ with probability p . Thus

$$\begin{aligned} E[Z^2] &= (1-p)E[A^2] + p * E[(A+B)^2] \\ &= (1-p)E[A^2] + p * E[A^2 + 2AB + B^2] \\ &= E[A^2] - p * E[A^2] + p * E[A^2] + 2 * p * E[A] * E[B] + p * E[B^2] \\ &= E[A^2] + 2 * p * E[A] * E[B] + p * E[B^2] \end{aligned}$$

4. $A - p * B$

Similarly, subtracting a fraction p of B from A results in:

$$\begin{aligned} E[Z] &= E[A] - p * E[B] \\ E[Z^2] &= E[A^2] - 2 * p * E[A] * E[B] + p * E[B^2] \\ E[Z^3] &= E[A^3] - 3 * p * E[A^2] * E[B] + 3 * p * E[A] * E[B^2] - p * E[B^3] \\ E[Z^4] &= E[A^4] - 4 * p * E[A^3] * E[B] + 6 * p * E[A^2] * E[B^2] - 4 * p * E[A] * E[B^3] + p * E[B^4] \end{aligned}$$

C. Moment calculations for mutually exclusive interventions

1. $A - p_1 * B_1 - p_2 * B_2 - p_3 * B_3 - p_4 * B_4$

The function in the model that calculates the resulting moments after applying mutually exclusive interventions (which in the model are the physical and chemical interventions before and after chiller), subtracts fractions of four set of moments from the set of moments before applying the interventions.

Let $\mu'_{k,A} = E[A^k]$ with $k = 1, 2, 3, 4$ be the raw moments of variable A (moments before applying mutually exclusive interventions) and let $\mu'_{k,B_i} = E[B_i^k]$ with $k = 1, 2, 3, 4$ be the raw moments of variables B_i (moments of the interventions), then the raw moments for variable $Z = A - p_1 * B_1 - p_2 * B_2 - p_3 * B_3 - p_4 * B_4$ are equal to:

$$\begin{aligned} E[Z] &= E[A] - p_1 * E[B_1] - p_2 * E[B_2] - p_3 * E[B_3] - p_4 * E[B_4] \\ E[Z^2] &= \\ &E[A^2] - 2 * E[A] * p_1 * E[B_1] - 2 * E[A] * p_2 * E[B_2] - 2 * E[A] * p_3 * E[B_3] - 2 * E[A] * p_4 * E[B_4] \\ &- 2 * p_1 * E[B_1] * p_1 * E[B_1] + p_1^2 * E[B_1^2] + p_2^2 * E[B_2^2] + p_3^2 * E[B_3^2] + p_4^2 * E[B_4^2] + 2 * p_1 * E[B_1] * p_2 * \\ &* E[B_2] + 2 * p_1 * E[B_1] * p_3 * E[B_3] + 2 * p_1 * E[B_1] * p_4 * E[B_4] + 2 * p_2 * E[B_2] * p_3 * \\ &E[B_3] + 2 * p_2 * E[B_2] * p_4 * E[B_4] + 2 * p_3 * E[B_3] * p_4 * E[B_4] \\ E[Z^3] &= E[A^3] - \dots + 6 * E[A] * p_3 * E[B_3] * p_4 * E[B_4] \\ E[Z^4] &= E[A^4] - \dots \end{aligned}$$

$$2. A + p_1 * B_1 + p_2 * B_2 + p_3 * B_3 + p_4 * B_4$$

In the inverse situation, where we know the set of moments after applying the mutually exclusive moments and we need to know the set of moments before applying them, we can just use the equations from above, as in this situation we know $E[Z^k]$ and we need to know $E[A^k]$.

$$E[A] = E[Z] + p_1 * E[B_1] + p_2 * E[B_2] + p_3 * E[B_3] + p_4 * E[B_4]$$

$$E[A^2] = E[Z^2] + \dots$$

$$E[A^3] = E[Z^3] + \dots$$

$$E[A^4] = E[Z^4] + \dots$$

D. Fitting distributions to moments

For this model we fit distributions of log cfus on a carcass by the first three central moments μ (Mean), V (Variance) and S (Skewness) according to following rules:

If $|Skewness| \leq 0.01$

Fit to a Normal(μ , SQRT(V)) distribution with mean μ and variance V

If $Skewness > 0.01$

Fit to a shifted Gamma distribution: $\delta + \text{Gamma}(\alpha, \beta)$ where:

$$\alpha = (2 / Skewness)^2$$

$$\beta = \text{SQRT}(Variance / \alpha)$$

$$\delta = \mu - \alpha * \beta$$

If $Skewness < -0.01$

Then fit to an inverted Gamma distribution: $\delta - \text{Gamma}(\alpha, \beta)$ where:

$$\alpha = (2 / Skewness)^2$$

$$\beta = \text{SQRT}(Variance / \alpha)$$

$$\delta = \mu + \alpha * \beta$$

E. Adjusting between flock prevalence (BFP) and within flock prevalence (WFP)

1. Factor k_{i+1}

If an intervention has an impact on the flock prevalence (between or within), **and data are available to estimate the effect on prevalence**, then the new prevalence is calculated based on the following formula:

$$\frac{p_{i+1}}{1 - p_{i+1}} = k_{i+1} \frac{p_i}{1 - p_i}$$

where p_i and p_{i+1} are the prevalence before and after applying the intervention and where k_{i+1} is the factor that represents the change in prevalence. The above formula can also be written as:

$$\text{logit}(p_{i+1}) = \text{logit}(p_i) + \log(k_{i+1})$$

This means that if, for a certain intervention X , we know what the prevalence was before (p_i) the intervention and after (p_{i+1}) the intervention, we can calculate the factor k_{i+1} as following:

$$k_{i+1} = \frac{p_{i+1} * (1 - p_i)}{p_i * (1 - p_{i+1})}$$

This factor can then be used in the model to calculate the prevalence q_{i+1} after applying intervention X when the prevalence before applying it is q_i :

$$q_{i+1} = \frac{k_{i+1} * q_i}{1 - q_i + k_{i+1} * q_i}$$

2. Example

Figure 21 shows examples of the relationship between p_i and p_{i+1} for various observed $\{p_i, p_{i+1}\}$ values shown as red markers. For example, the top curve would apply if one observed a change in prevalence from 50% to 40% for some particular intervention: the formula would then predict that applying the same intervention where the original prevalence is 20% would reduce the prevalence to about 14% (yellow marker).

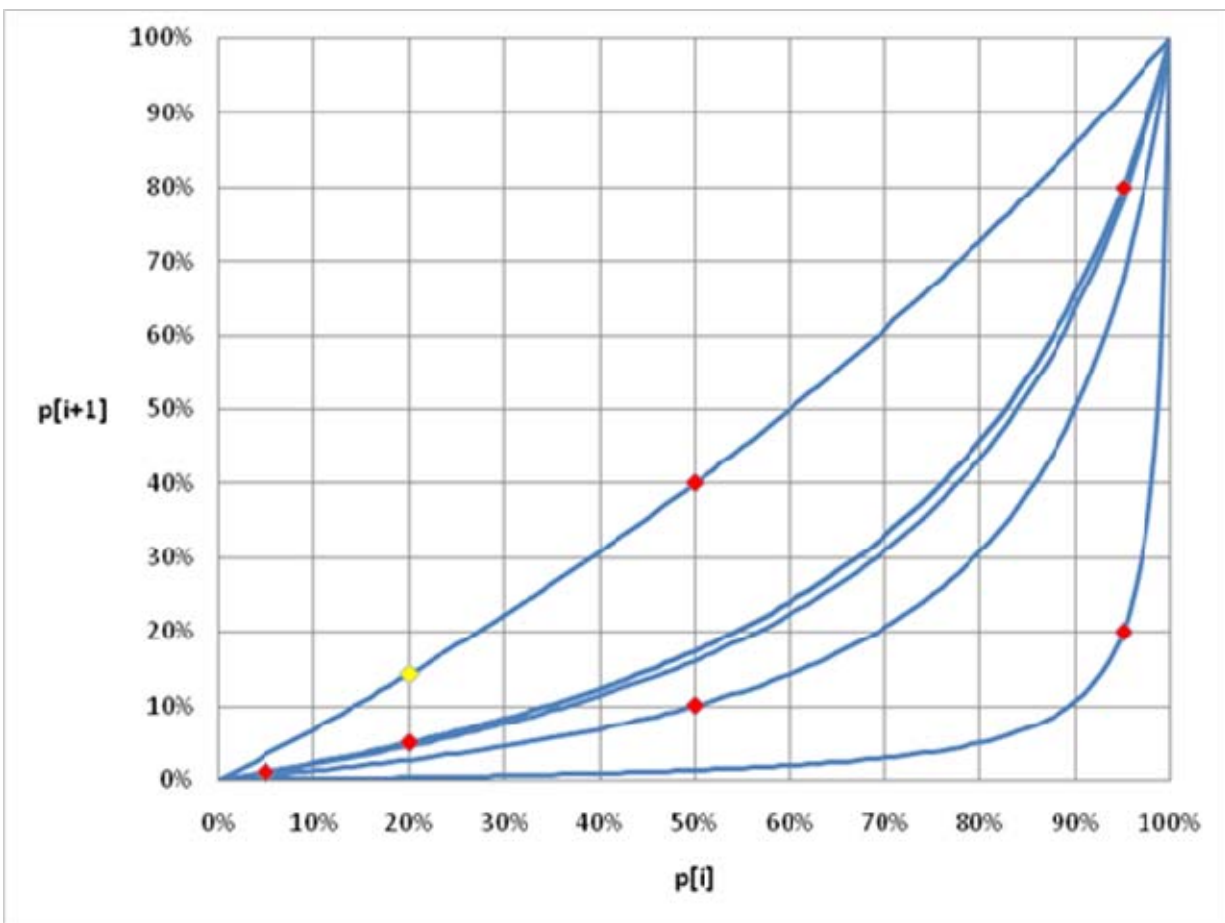


Figure 21 Relationship between $p[i]$ and $p[i+1]$

3. Subjective estimates

The model allows the user to input *subjective estimates* of the change in prevalence if no data are available. In this case, using the k-factor approach above would be inappropriate since it would require

the expert to estimate k , which is not an intuitive variable to conceive of. For subjective estimates, the user is therefore asked to estimate what the proportional reduction r will be on the prevalence if an intervention is applied to all flocks. For example, if flock prevalence was 40% and the user gave a value $r = 25\%$, then the new prevalence would be estimated as $40\% \cdot (1 - 25\%) = 30\%$.

The forward and backward estimations of between flock prevalence are as follows:

$$BFP_a = fBFP_b(1 - r) + (1 - f)BFP_b = BFP_b(1 - fr)$$

$$BFP_b = \frac{BFP_a}{(1 - fr)}$$

Where BFP is the between flock prevalence, b , a are subscripts for before and after an intervention respectively, and f is the fraction of flocks to which the intervention is applied.

For intervention affecting within-flock prevalence (WFP), we have only forward calculations since the model assumes that the within flock prevalence is 100% when a flock is infected:

$$WFP_a = WFP_b(1 - fr)$$

F. Statistical interpretation of data

The model has been constructed so that it allows the user to estimate moments and parameters from data either deterministically (best estimate) or stochastically (including statistical uncertainty). These two methods are described for the different data sets here below.

1. Central moments estimation

This is about estimating the central moments representing the log-load distributions. In *Deterministic* mode, the model uses the sample moments; and in *Stochastic* mode, the model applies Non-Parametric Bootstrapping by using ModelRisk's *VoseNBootMoments()* function. The principle of this is that it resamples from the data set and recalculates the central moments of the resampled data to give samples from the joint uncertainty distribution for the central moments.

2. Prevalence or probability uncertainty

Where a data set has n independent trials, of which s are “successes”, the probability of success is:

Deterministic mode: s/n

Stochastic mode: Sample from a Beta($s+1$, $n-s+1$) distribution

3. Data where mean and mean standard error or confidence intervals only are available

a. Case 1: Mean (\bar{x}) is provided with mean standard error (MSE) as well as the number of samples n

Deterministic mode:

$$\mu = \bar{x}$$

$$V = n * MSE^2$$

$$S = 0$$

$$K = 3$$

Stochastic mode:

$$\mu = \text{Normal}(\bar{x}, \text{MSE})$$

$$V = (n-1) * n * \text{MSE}^2 / \chi^2(n-1)$$

$$S = 0$$

$$K = 3$$

b. Case 2: Mean (\bar{x}) is provided with $(1-\alpha)$ confidence interval around mean ($\pm \delta$) as well as the number of samples n

In this case, δ is translated to a MSE value. For example, at $\alpha = 5\%$ (i.e. a 95% confidence interval) this equates to $1.96 * \text{MSE}$ and thus $\text{MSE} = \delta / 1.96$. The formulae in Case 1 are then applied.

4. Data where mean (\bar{x}) and variance (s^2) and number of samples n are available

Deterministic mode:

$$\mu = \bar{x}$$

$$V = s^2$$

$$S = 0$$

$$K = 3$$

Stochastic mode:

$$\mu = \text{Normal}(\bar{x}, \text{SQRT}(V/n))$$

$$V = (n-1) * s^2 / \chi^2(n-1)$$

$$S = 0$$

$$K = 3$$

G. Slaughter age – Logistic regression

1. Slaughter age and thinning as interventions

Based on the EFSA Baseline data set, we can look up for each country the relationship between the age of slaughter of each sample chicken and whether or not that sample was infected. Typically, we will expect to see more positive samples for chickens that were slaughtered at a later age. This means that lowering the slaughter age can have an effect of reducing the between flock prevalence (BFP). The new maximum slaughter age to be used in a scenario is input at Row 42 of the model in the red cell:

Contamination/prev		
Slaughter age	Max observed age in current state:	42
	Max desired age in future state:	

Similarly, we can look at the relationship between the proportion of farms that apply thinning in a country and the prevalence in that country. The WG advised that thinning is only applied in indoor flocks and the model was constrained appropriately. The new proportion of farms applying thinning is entered at Cell AP36:

Contamination/prev		
Thinning	Proportion applied in current state:	24.9%
	Proportion applied in future state:	

2. Logistic regression

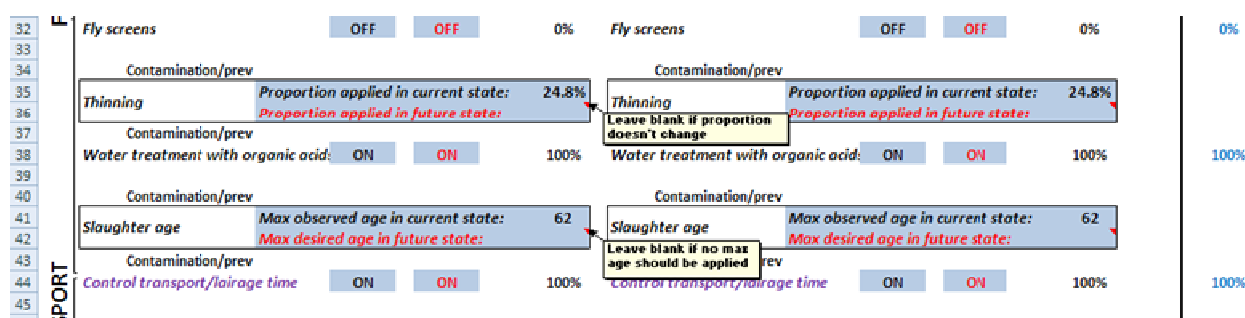
As mentioned above, we used the EFSA Baseline data set to estimate the relationship between slaughter age and thinning, and prevalence. This was done by using the parameter estimates of a logistic regression performed by a contractor to EFSA. The formula for the logistic regression applied in this model is of the form:

$$\ln\left(\frac{p}{1-p}\right) = k + \beta_1 * age + \beta_2 * thinning + \varepsilon$$

where p is the prevalence, age is the average age of slaughter, $thinning$ is the proportion of thinning applied, β_1 and β_2 are two regression coefficients, k is a constant specific to each country and ε is an error term equal to a Normal distribution with mean 0 and standard deviation σ . Once we know the constant k for each country by fitting to the data set, the above equation will give us the new prevalence if the average age of slaughter is lowered or the percentage of applied thinning is changed in a certain country. The average age of slaughter can be lowered in the model by specifying a maximum slaughter age (which is the control for the user to select if s/he chooses to include this intervention). The maximum slaughter age specified by the user works as a truncation of a LogNormal distribution fitted to the original data set of slaughter ages for each country separated out by indoor and outdoor flocks. This truncated LogNormal distribution now has a new (and lower) mean that can be calculated using the following formula:

$$Mean_{New} = \left\{ \int_0^{Max_{age}} x * f(x) dx \right\} * F(Max_{age}) + \left[(1 - F(Max_{age})) * Max_{age} \right]$$

where $f(x)$ and $F(x)$ are the probability density function and the cumulative distribution function of the LogNormal distribution fitted to the data set for a particular country. This new mean is then used in the logistic regression equation as shown above, together with the country-specific constants to give us the new prevalence as a result of lowering the maximum slaughter age. Similarly for thinning, the percentage of applied thinning can be lowered in the model, which will then result in a new prevalence based on the logistic regression equation as shown above.



The above Figure is a screen capture of the section in the sheet 'Country' in the model's Future State where a maximum slaughter age or a new thinning proportion can be entered

The values for the age regression coefficient β_1 , the percentage thinning regression coefficient β_2 and the standard deviation of the logistic regression σ can be changed (cells shown in green) in the 'Logistic regression' section of the 'Inputs & Control' sheet as shown in the Figure below.

	A	B	C	D	E	F	G	H
192								
193								
194								
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203								

Logistic regression: Slaughter age and Thinning

Age regression coefficient	β_1	0.06742
% thinning regression coefficient	β_2	0.5521
Std dev of logistic regression	σ	0

				Slaughter age			
	Name	Letters	Code	Min	Max	Mean	StDev
	Austria	AT	1	26	65	35.33	3.85
	Belgium	BE	2	33	79	40.90	4.43