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Toxoplasma infections through meat in the Netherlands

Risk assessment update

RIVM report 2025-0013
A.N. Swart | M. Opsteegh

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Colophon

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A.N. Swart (author), RIVM
M. Opsteegh (author), RIVM

Contact:
Arno Swart
Centre for Infectious Disease Control
arno.swart@rivm.nl

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Synopsis

Toxoplasma infections through meat in the Netherlands

Risk assessment update

People can become infected with the parasite *Toxoplasma* through food. Although the infection will not make most people ill, it can pose a risk to unborn children during pregnancy. Infection could also pose a risk to people with severely impaired immunity, such as people with AIDS or people who recently had an organ transplant.

Therefore, it is important to understand the major sources of infection. This knowledge is needed for health counselling, for example, by doctors and midwives. It also allows for action to be taken to reduce the risk of becoming infected through those sources.

The infection risk is calculated using a QMRA model. RIVM has now updated this calculation model for the second time. This update concerned infections through meat and was prompted by the availability of more and more recent data. The initial model dated from 2011 and was first updated in 2020.

The parasite is spread by cats. Oocysts (a type of small parasite eggs) end up in the environment – and ultimately in products such as fruit and vegetables – through cat poop. When these contaminated products are not properly washed, peeled or sufficiently cooked, they can cause infections in people. When animals are infected, parasites end up in their muscles and ultimately in their meat. If meat from animals such as cows and pigs is not frozen or is consumed while still undercooked, it can cause an infection.

For fresh meat, such as steak or pork tenderloin, the risk of infection per prepared portion is usually low. However, as these products are widely consumed, fresh meat is a major source of *Toxoplasma* infections when the risk is calculated for the entire population. This is relevant information for policymakers.

The highest risk of infection for individuals is when they consume raw meat products, such as steak tartare, rosbief and filet americain. The risk of infection through raw meat products largely depends on the salting process during production. Salt reduces the chances of a parasite surviving the production process. The extent of the effect of salting the products remains uncertain in the model. The new insights into this matter will be incorporated into the next update.

Keywords: QMRA, *Toxoplasma gondii*, toxoplasmosis, risk assessment

Publiekssamenvatting

Toxoplasma-infecties via vlees in Nederland

Update risicoschatting

Via voedsel kunnen mensen besmet worden met de parasiet Toxoplasma. De meeste mensen worden daar niet ziek van, maar tijdens een zwangerschap kan een infectie gevaarlijk zijn voor het ongeboren kind. Ook voor mensen met een ernstig verzwakte afweer kan een besmetting gevaarlijk zijn, zoals mensen met aids of mensen die net een orgaantransplantatie hebben ondergaan.

Het is daarom belangrijk om te weten wat de belangrijkste bronnen van een besmetting zijn. Deze informatie is nodig om goede voorlichting te kunnen geven, bijvoorbeeld door artsen en verloskundigen. Ook kunnen daarmee maatregelen worden genomen die de kans op een besmetting via deze bronnen kan verminderen.

De kans op een besmetting wordt met de zogenoemde QMRA-modellering berekend. Het RIVM heeft dit rekenmodel voor de tweede keer geüpdatet. Dat is voor infecties via vlees gedaan, omdat hierover meer en recentere data beschikbaar waren. Het oorspronkelijke model dateert uit 2011 en is in 2020 voor het eerst geüpdatet.

Katten zijn de belangrijkste verspreiders van deze parasiet. Via kattenpoep komen oöcysten, een soort eitjes van de parasiet, in de omgeving terecht, en zo bijvoorbeeld ook op groente en fruit. Als mensen deze besmette producten eten terwijl ze niet goed zijn gewassen, geschild of verhit, dan kunnen ze een infectie krijgen. Als dieren de infectie krijgen, komen er parasieten in de spieren terecht, en vervolgens in het vlees. Als vlees van bijvoorbeeld koeien en varkens niet wordt ingevroren en onvoldoende verhit wordt gegeten, kan het een infectie veroorzaken.

Voor vers vlees, bijvoorbeeld biefstuk of varkenshaas, is de kans op een besmetting per bereide portie meestal klein. Maar omdat deze producten veel worden gegeten, is vers vlees een belangrijke bron van Toxoplasma-infecties als de kans voor de hele bevolking wordt berekend. Deze informatie is voor beleidsmakers relevant.

Op individueel niveau is de kans op een infectie het grootst als mensen rauwe vleesproducten eten, zoals steak tartaar, rosbief en filet americain. Bij rauwe vleesproducten hangt de kans op infectie er vooral van af hoeveel het vlees tijdens de productie gezouten is. Zout verkleint de kans dat een parasiet overleeft. Hoe groot het effect van het zouten is, is in het model nog onzeker. De nieuwe inzichten hierover worden in een volgende update verwerkt.

Kernwoorden: QMRA, *Toxoplasma gondii*, toxoplasmose, risicoschatting

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Summary

Toxoplasma gondii is an important foodborne pathogen with many potential sources of infection. At RIVM source attribution for meatborne *T. gondii* infections is based on a quantitative microbial risk assessment model initially published in 2011 and first updated in 2020. The work presented in this report builds on experiences from previous models for *T. gondii* infection risk from meat consumption. Improvements have been make with regard to input data and conceptualization of the submodels. All input data (prevalence in animals, anatomical distribution of the parasites in the hosts, consumption data, meat processing data), as well as submodels (inactivation by heating, salting, freezing and a dose-response model) were combined in the final framework of the QMRA model to obtain outputs for a wide range of meat-products. The developed model takes into account uncertainty in fitted model parameters (e.g. inactivation efficiency, consumption rates) and natural variation. The processing of the products was based on gathered literature information and modelled using inactivation models.
Since infection with *T. gondii* is assumed to persist lifelong, 'age at first infection' and associated variables like the product, animal species, and processing steps, were chosen as outcomes for the exposure assessment. Using the age at first infection has the additional benefit that direct comparison with human seroprevalence data is possible. Source attribution was performed by comparing the contributions of different products to the modelled first infections.

At species and product level, the importance of beef and filet americain[1] is consistent between this QMRA model and the two previous ones. At product level, rosbief and steak tartare present a higher risk than filet americain and, taking consumption into account, at population level too, rosbief is now estimated to be slightly more important than filet americain. For meat products such as filet americain and rosbief, which are processed and bought ready to eat, it is important to take into account that there is uncertainty in the processing parameters and the effect of salting on *T. gondii* viability.

One important difference compared to the previous QMRA models is the increased importance of fresh meat products, which is now estimated as the most important source of infections at population level. Consumption of fresh meat products was and still is high. The reason for this increase is that the model for the effect of heating on *T. gondii* viability has been modified. Of course, for fresh meat products, the risk for individual consumers depends heavily on preparation habits, and infection can be prevented by freezing or properly heating these products.

[1] See the Glossary for an explanation of Dutch speciality products

1 Introduction

Toxoplasmosis is a zoonotic disease caused by the single-celled parasite *Toxoplasma gondii*. Although most infections are asymptomatic in immunocompetent individuals, the infection can cause severe health problems in pregnant women and immunocompromised patients (Montoya & Liesenfeld, 2004). In the Netherlands, it was estimated that 30% of the general population is seropositive (van den Berg et al., 2023) and the incidence of congenital toxoplasmosis was estimated at 2 cases per 1000 live born children (Kortbeek et al., 2009). In addition, *T. gondii* is associated with one of the highest disease burdens among fourteen food-related pathogens in the Netherlands (Benincà et al., 2024). Humans acquire *T. gondii* infection through consumption of raw or undercooked meat containing viable tissue cysts, or through ingestion of oocysts present, for example, in soil, water or on contaminated fruits and vegetables (Tenter et al., 2000).

The EJP One Health project TOXOSOURCES addressed the research question – *What are the relative contributions of the various sources of T. gondii infection?* – using several multidisciplinary approaches as well as novel and improved methods to yield estimates that can inform risk management and policy-making. The main outcomes of TOXOSOURCES were: a quantitative estimate of the contribution of the main sources and transmission routes of *T. gondii* infection based on improved quantitative microbiological risk assessment (QMRA) models (Work Package (WP) 2); new data filling the key knowledge gap about the role of increasingly popular but unstudied ready-to-eat (RTE) fresh produce (WP3); a novel serological method that aims to specifically detect infections caused by oocysts (WP4); a novel typing method to detect introduction of atypical *T. gondii* strains (WP5). WP2 on QMRA was coordinated by RIVM. Associated with this EJP project, ANSES and RIVM co-coordinated the OHEJP PhD project ToxSauQMRA, focusing on pork products in France.

TOXOSOURCES WP2 'Multicentre quantitative microbiological risk assessment for *T. gondii* infections' aimed to quantify the relative contribution of sources of *T. gondii* infection, including meat products, fresh produce and environmental pathways, in all EU regions by QMRA. The main routes of infection involve tissue cysts (meat) and oocysts (environmental pathways). These models apply to nine countries representing all four EU regions.
Input data for the QMRA was collected by all partners and WP3. An overview of the prevalence of *T. gondii* infection in humans, animals used for human consumption, as well as in cats, was obtained by reviewing the available literature, including grey literature. Exposure data was collected in a harmonised way using a survey specifically designed for QMRA purposes. Region- or country-specific products, dishes or eating habits were identified and the associated processing parameters were collected by the partners.

In this report, we apply the TOXOSOURCES model to the Dutch situation, for the case of meatborne infection, thereby presenting an

update to the earlier QMRA studies by Opsteegh et al. (2011) and Deng et al. (2020). The results of the TOXOSOURCES model will be compared to the earlier results, and updated risk ranking of products will be presented.

2 Previous QMRA models

For the Netherlands, two QMRA models for *T. gondii* infections have been published previously, the study by Opsteegh et al. (2011), followed by the study by Deng et al. (2020), which updated the 2011 study using new data.

2.1 The 2011 QMRA model

This QMRA model was the first quantitative risk assessment for meatborne *T. gondii* infections in the Netherlands. It includes all relevant animal species, but the parasite load was based on sheep heart only. The processing steps considered in the model were: salting, freezing, and heating. Consumption data was taken from the Dutch national food consumption survey. As a dose-response model for humans was – and is – unavailable, a mouse dose-response was used instead. To account for pre-existing immunity, a reduction factor was applied to the number of infections, based on serological infection estimates. An overview of this model, and a comparison with the models that follow, can be found in Table 1.
The model predicted that beef and veal were by far the highest ranked in the attribution: 68% of all cases were estimated to originate from beef consumption. The absolute number of cases per year was extremely high at almost four million (Table 6).

2.2 The 2020 QMRA model

The model by Deng is an update of the 2011 model. One important difference with the previous model is that the parasite load for cattle was reduced 100-fold, on the basis of new insights. Moreover, the effect of salting was updated using more data from literature. Also, at the consumer side, the data was updated to the most recent consumption data at that time, and consumption behaviour was also updated to newly available literature information (Table 1).

Table 1 Comparison of the three QMRA models.

	2011 QMRA model	***2020 QMRA model***	***Toxosources QMRA***
Scope			
Country	The Netherlands	The Netherlands	Nine EU countries
Primary Production			
Animal prevalence	Infection prevalence from studies carried out in the Netherlands Not age-dependent, no uncertainty	Update for prevalence in pigs and in cattle	Age-dependent infection prevalence model fitted at European region level, to data from literature. Includes uncertainty
Parasite load	Based on heart samples of naturally infected sheep Animal species not differentiated Anatomical differences not considered Variation of load over portions, with mean 3981 bradyzoites per 100g	As in 2011, except the load for beef and veal which was reduced 100-fold	Based on literature study Six animal species differentiated Added probability that tissue is positive in an infected animal differentiated by organ/heart/muscle Variation of load over portions
Processing			
Product properties	Several literature sources	New data from Evers et al. (2017)	New processing table from several literature sources and input by experts from the various countries
Salting	Based on one inactivation study based on mouse bioassay (Dubey, 1997b) Logistic regression for probability of infection, inverse dose-response	More data from literature added to fit the model	Conceptually improved model, extended data as compared to the Deng et al. (2020) model
Freezing	Only by consumers Probability of freezing by consumers differentiated into two	Both consumers and producers	Both consumers and producers Complete inactivation assumed

	2011 QMRA model	***2020 QMRA model***	***Toxosources QMRA***
	product groups and four freezing temperatures. Freezing time follows an uniform distribution of 1-30 days Model as in salting phase, based on data from Kotula et al. (1991)	Probability of freezing by producers differentiated into four categories depending on product type and at -18 or -24 °C Probability of freezing by consumers differentiated into four product groups with temperature and time as in Opsteegh et al. (2011) Model same as in 2011	
Heating	Two product categories with different heating temperature distribution Model as in salting phase, based on data from Dubey et al. (1990) but only core temperatures directly after heating used and no time effect considered	Ten product categories with different heating profile based on Evers et al. (2017) Model same as in 2011	Extended model including time effect and heat diffusion by product shape, based on the complete dataset from (Dubey et al., 1990)
Consumption Phase			
Products	Pork, beef, sheep. Relevant products (n=50) selected from those reported in the consumption survey	Pork, beef, mutton. Relevant products (n=83) selected from those reported in the consumption survey	Selection of potentially risky meat and vegetable products. Includes soil
Consumption Frequencies	(Anonymous, 1998) No variability Sex, age not differentiated	VCP 2007-2010 (van Rossum et al., 2011)	Toxosources Survey Variability over population Sex, age differentiated
Portion size	(Anonymous, 1998) Variability over population Sex, age not differentiated	VCP 2007-2010 (van Rossum et al., 2011)	Toxosources Survey Variability over population Differentiated by sex and age

	2011 QMRA model	***2020 QMRA model***	***Toxosources QMRA***
Risk Characterisation			
Dose Response	Based on mouse data No uncertainty	Same as 2011	Based on several non-human mammalian species Includes uncertainty
Population Risk	Mean probability of illness per product times the number of portions consumed of this product	Same as 2011	Per-individual simulation, with person specific consumption pattern
Immunity	Application of a fixed 'immunity factor'	Same as 2011	Only the first infection per person is counted

3 The Toxosources QMRA model

In this section, we will follow the structure of Table 1, and compare the Toxosources QMRA to the previous models from 2011 and 2020. At each stage, we will highlight and explain the changes with respect to the previous models.

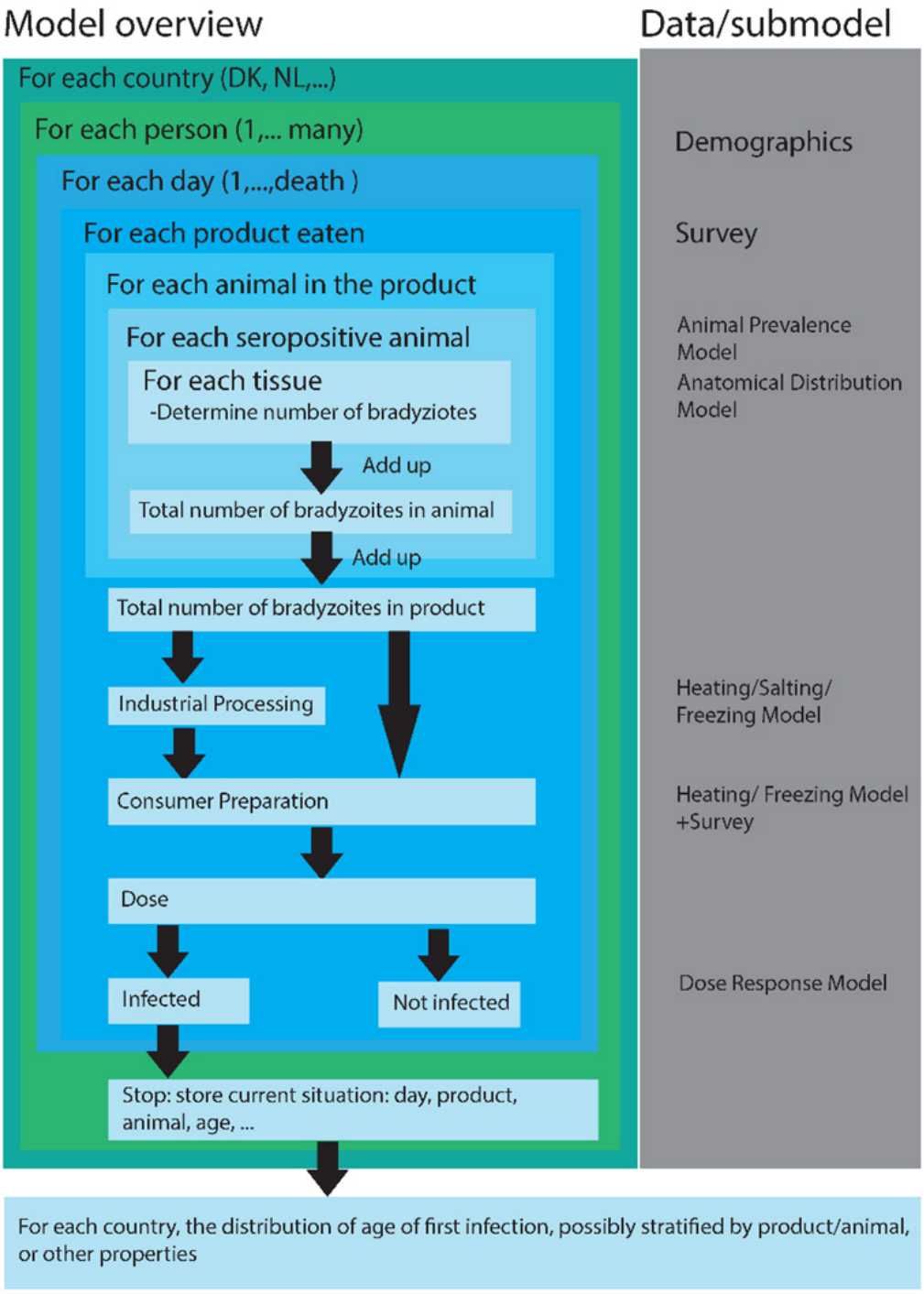
3.1 Scope

The Toxosources QMRA was built for nine European countries, and involves multiple transmission routes: meatborne (via the bradyzoite form of *T. gondii*), via vegetables (via the oocyst form of *T. gondii*) and via soil (also via oocysts). In this report, we focus on the meatborne route for the Netherlands, as we wish to compare it to the previously published QMRA models.

3.2 Model Structure

The model is structured as schematically shown in Figure 1. The model is built with several nested layers: for each country (only the Netherlands in this report), we simulate 2500 persons. For each person, we simulate each day up to age 80. For each day, we determine which products are consumed, and for each product, we consider each animal used in the product (mostly one animal, but some products are composite). For each seropositive animal, we consider all positive tissues. Subsequently, all organisms from all animals are added up. In the next steps, the number of bradyzoites is followed throughout the chain. First, there are several processing and consumer handling steps, such as heating, salting, and freezing, which are outlined in the following subsections. Finally, an individual person might receive a dose of bradyzoites if any survived the chain, and a dose-response relation (Section 4.8) determines whether infection occurs.
Throughout this model, we keep records of all individuals, products, animals, parasite loads, and ages of infection, which enables us to derive age-dependent infection probabilities and attribution of infection to sources at a later stage.

Figure 1 Model structure of the Toxosources QMRA. Nested boxes indicate nested model compartments. Arrows indicate the direction of the food chain, and the boxes are in the order in which the model steps are performed. The rightmost column indicates what data sources or sub-models were used.



3.3 Animal prevalence

In the 2011 QMRA, prevalence estimates, obtained from our own studies or from literature, were used without uncertainly estimates. For pigs, a weighted average of the seroprevalence in slaughtered organic and conventional pigs was used. Separate prevalence estimates for sheep and lamb were applied. Sheep had by far the highest prevalence (Table 2).The 2020 QMRA utilized new data for cattle and pigs. In both publications, the authors argue that direct rather than indirect (antibody) detection results should be used for cattle. In the 2011 model the prevalence of *T. gondii* infection in cattle is based on PCR data and in the 2020 model on data obtained by mouse bioassay.

For the Toxosources QMRA, data were needed for all nine EU countries included and therefore an extensive literature search was performed for animal prevalence data. Instead of averaging the prevalences found, an age-dependent model was used (Damek, Swart, et al., 2023), as infection accumulates with age. In the model, several EU regions were defined; for the current report we use the region 'West', which includes the Netherlands. Furthermore, a distinction between indoor and outdoor was introduced, as animals with outdoor access are more likely to be exposed to the parasite. Also, uncertainty was quantified in the estimates. Similarly to the previous QMRA models, data based on direct detection methods were used for cattle. We find that all prevalences are higher than in the previous QMRAs, particularly regarding lamb and sheep.

Table 2 Prevalence of T. gondii infection by livestock species as used in the models. Confidence intervals (95%) are indicated in square brackets.

Species	2011 QMRA	2020 QMRA	Toxosources
Cattle	2%	1.6%	3.4% [2.4%, 4.6%]
Pigs	0.5%	2.0%	8.5% [7.9%, 9.1%]
Lamb	17.7%	17.7%	43.0% [40.8%, 45.0%]
Sheep	53.2%	53.2%	79.3% [77.1%, 81.3%]
Chicken	-	-	9.7% [8.6%, 10.9%]

3.4 Parasite load

In the 2011 QMRA, the parasite load was based on qPCR-results for heart samples from naturally infected sheep. These estimates were used for all species and anatomical parts. The variation of the load in meat per gram was described by a distribution of log-10 transformed loads with mean 1.6 and 95% confidence interval [-0.2, 3.4].
In the 2020 QMRA this value was updated for cattle only, by reducing the load 100-fold.
In the Toxosources QMRA, we explicitly considered both the probability of a tissue being positive (Table 3) and the parasite load for a positive tissue (Table 4). The fact that not all tissues in an infected animal are positive had not been considered in the previous QMRA models. As validation, the load for sheep heart found in the Toxosources model matches very well with the 2011 QMRA. The 100-fold difference in load between sheep and cattle that was applied in the 2020 model matches reasonably with the results currently obtained for these species (Table 4). However, using concentrations based on heart rather than muscle and disregarding the fact that not all tissues of a positive animal contain

parasites resulted in a higher parasite load in the previous models compared to the Toxosources QMRA.

Table 3 The probabilities of tissues being positive in an infected animal with 95% confidence interval.

animal	heart	muscle	organ
chicken	0.6 [0.5,0.7]	0.5 [0.4, 0.6]	0.6 [0.5, 0.7]
pig	0.8 [0.7, 0.9]	0.6 [0.5, 0.6]	0.5 [0.4, 0.6]
sheep	0.9 [0.8, 1.0]	0.7 [0.6, 0.8]	0.4 [0.2, 0.5]
cattle	0.2 [0.1, 0.6]	0.3 [0.2, 0.4]	0.1 [0.0, 0.4]

Table 4 Log-10 transformed bradyzoite load per gram in positive tissues with 95% confidence interval.

animal	heart	muscle	organ
chicken	3.2 [1.1, 5.3]	2.3 [-0.45 4.5]	1.8 [-0.9, 4.4]
pig	0.6 [-1.8, 2.6]	-0.7 [-3, 1.7]	-1 [-3.5, 1.9]
sheep	1.3 [-0.8, 3.6]	0.4 [-2.1, 3]	-0.1 [-2.7, 2.6]
cattle	-0.4 [-2.7, 2.1]	-1.4 [-3.9, 0.7]	-1.7 [-4.2, 0.8]

3.5 Product properties

The 'product properties' category comprises all parameters relevant to the products being considered. This includes processing parameters (for example, times and temperatures for heating, duration, and NaCl percentage for salting, frequency of freezing of portions, etc.), but also the proportion of the product that is meat.

The differences between the models are too numerous to be exhaustively listed here. Each QMRA model is based on information derived from various sources.

Comprehensive information on the processing details for a wide variety of meat products is needed to realistically reflect the potential inactivation of the parasite. Therefore, temperatures, processing times, salt concentrations in combination with salting methods, and storage conditions used during production were obtained from previously described sources (including European and national regulations, guild standards, specialised literature, scientific publications, cookbooks, traditional recipes, and product labels). The spreadsheet containing all processing settings can be accessed at https://github.com/rivm-syso/Toxosources.

3.6 Inactivation models

3.6.1 Background

Meat products are processed before consumption, and it is known that the various treatments affect *T. gondii* viability. Freezing the meat below -12 °C at its core for at least three days inactivates all *T. gondii* parasites (Kotula et al., 1991). Similarly, properly cooking meat and meat products at temperatures above 67 °C has been shown to be effective in inactivating *T. gondii* (Dubey et al., 1990). Previous studies showed a moderate effect of NaCl on reducing the viability of *T. gondii* in meat products (Damek, Fremaux, et al., 2023).

To accurately represent the reality of *T. gondii* inactivation in meat products, a robust model must account for multiple factors, such as duration and temperature during processing, and the NaCl content in the products. By incorporating experimental data into these inputs, the model can extrapolate to unmeasured scenarios, yielding a deeper understanding of variability, uncertainty, and consistency in experimental results. Importantly, a reliable model has the potential to replace the need for animal experiments. Previous models have attempted to assess the risk of *T. gondii* for consumers using quantitative microbial risk assessments, incorporating inactivation by salting and heating (Deng et al., 2020; Opsteegh et al., 2011). However, in this study, we developed a conceptually improved model using all available data from bioassay experiments.

3.6.2 Salting model development

Mouse bioassay data from salt inactivation experiments in literature only gives information on the probability of infection for mice, but in the QMRA model, the effect on the number of viable bradyzoites is needed. This was already clear when the salt inactivation model for the 2011 QMRA was developed (and used accordingly in the 2020 QMRA), but now, we realise that the approach used to overcome this issue was based on flawed reasoning, which proceeds as follows. First, assume a linear regression for the infection probability of mice, as a function of salting parameters (concentration, time, temperature etc.) $x_1, \dots, x_n$ with unknown parameters $\beta_0, \dots, \beta_n$,

$$\text{logit}\big(P_{\text{inf,mice}}(x)\big) = \beta_0 + \beta_1 x_1 + \cdots \beta_n x_n$$

The probability of infection in mice depends on the dose of infectious bradyzoites and, after processing, this dose depends on the processing parameters. However, what is left after processing also depends on the starting dose, which will differ across experiments and is not evaluated and reported in the literature. Moreover, there is a dose-response relation for the salting procedure and inactivation of bradyzoites as well as a dose-response relation for bradyzoite dose and infection in mice, and this combination is not handled properly in one logistic regression model (a linear relation between the salting parameters and log of the odds for mouse infection is assumed).
However, conceptually, while the salting parameters do not directly influence the number of mice infected, they do influence the presence of viable bradyzoites.
Then, the dose response relation for the probability of mice infection is applied:

$$P_{\text{inf,mice}} = 1 - e^{-rD} \rightarrow \ln(1 - P_{\text{inf,mice}}) = -rD$$

Then, a reduction factor is defined:

$$RF = \frac{D(\boldsymbol{x}_p)}{D(\boldsymbol{x}_0)} = \frac{\ln(1 - P_{\text{inf,mice}}(\boldsymbol{x}_p))}{\ln(1 - P_{\text{inf,mice}}(\boldsymbol{x}_0))}$$

Here, $\boldsymbol{x}_p$ are settings for salting parameters, and $\boldsymbol{x}_0$ are settings for when no salting is applied. However, this results in an undefined

denominator for $P_{\text{inf,mice}}(x_0) = 1$, given that $\ln(0)$ is undefined. Furthermore, the uncertainty in this approach is very large, since for a small probability of infection, the dose-response curve is very flat, and consequently, the dose corresponding to this probability becomes very uncertain. This is even more worrying when we consider that there are many parameter sets $\boldsymbol{x}_0$ for which we will have no effect, and consequently, a wide range of doses for which we will have no effect.

To circumvent these problems, we developed a new model that describes the effect of salt on bradyzoites (instead of on mice), and includes uncertainty in the estimates by means of Bayesian statistics. The model is outlined in Appendix A.

3.6.3 Heating model development

It is well known that by cooking a meat product well, the bradyzoites in it may become inactivated. Yet, the definition of 'well cooked' is not straightforward in the context of microbiological safety. It is a question on the degree of inactivation given a certain time and temperature of cooking. Franssen et al. (2021) proposed that inactivation of pathogens follows the amount of heat energy accumulated during the cooking event in that particular position. To make this calculation, we need to know the spatial temperature profile in a product at every time point during cooking and then integrate it in time. The temperature profile can be modelled by using the standard formula for heat diffusion. For sake of simplicity, we approximated the shape of the various products using standard geometrical shapes (i.e. meatballs as a sphere, sausages as a cylinder, and burgers or steaks as a slab). Depending on the geometry of the meat product being cooked, solutions for the heat diffusion equation can be calculated.

Under all geometries, we consider R to be the distance from the centre/core ($r = 0$) of the meat product to its surface. We assume that, across the meat product, the initial temperature is uniform: $T(r) = T_0, 0 \leq r \leq R$, and the boundary condition $T(R) = T_{\text{cooking}}$, i.e. the temperature of the outermost layer is the same as the temperature of the heating medium (oven, water, oil, etc.). We implemented the calculations for the three geometries in R (R Core Team, 2024). A value of the thermal diffusivity coefficient $\alpha = 0.0811\ mm^2/s$ was found to reproduce the temperature measurements carried out by Pesciaroli et al., (2019) well, as shown in Figure 2 and Figure 3.

Figure 2 Temperature over time in meatballs by distance to the centre. Solid lines indicate the modelled temperature over time for a meatball (at the indicated distance from the core). The joined points are the measurements carried out by Pesciaroli et al. (2019).

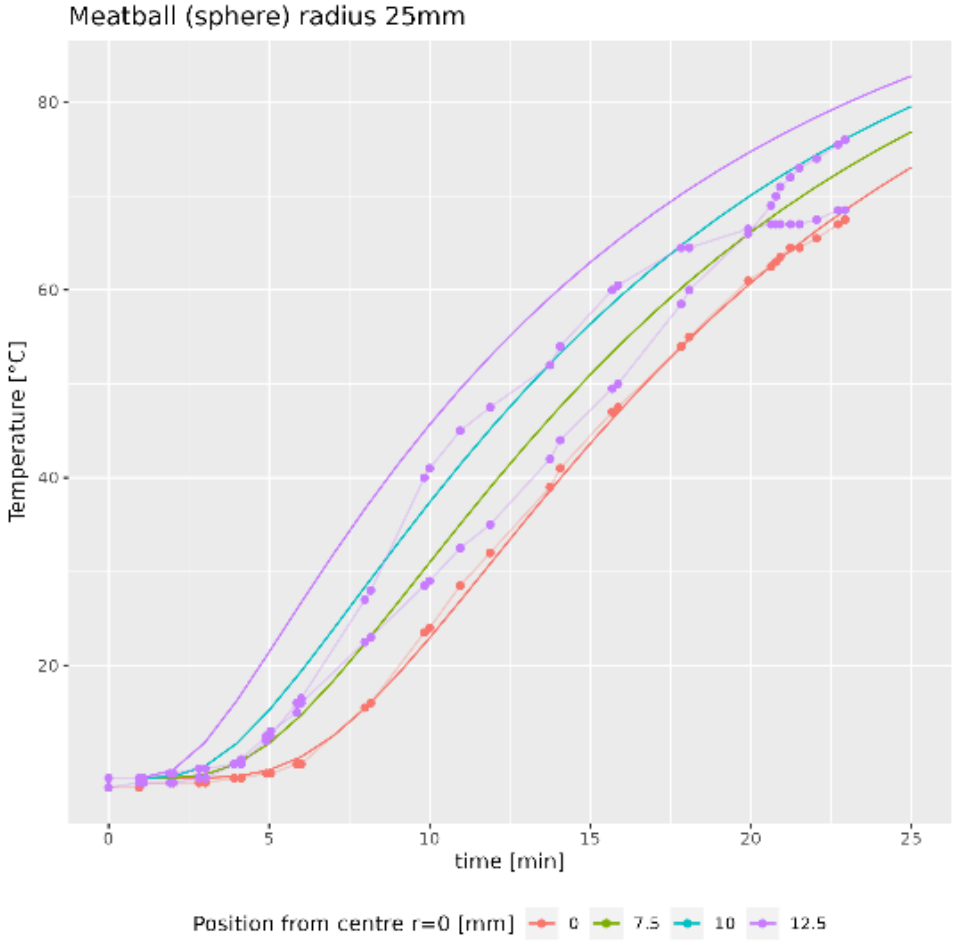
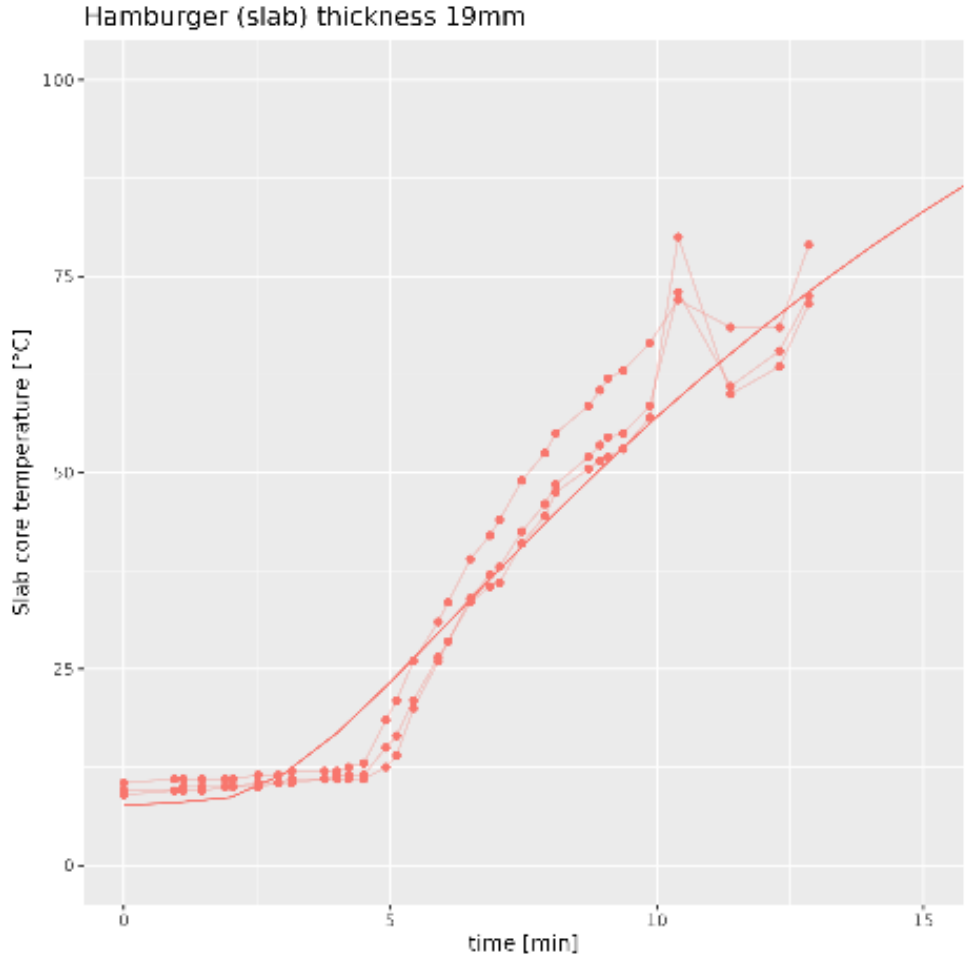


Figure 3 Temperature over time for a 19mm thick hamburger. Solid lines indicate the modelled temperature in over time for a hamburger at its core. The joined points are the measurements carried out by Pesciaroli et al. (2019).



Solutions are dependent on the input parameters (cooking time, heating temperature, initial temperature) and on the shape and size of the meat product.

3.6.3.1 Probability of inactivation at fixed temperature

The next step is to infer an inactivation rate dependent on temperature and time. In a set of trials, Dubey et al. (1990) carried out experiments measuring the probability of mice becoming infected after being fed pieces of meat cooked for various times, at various fixed temperatures. As explained in the section on the salting model, this probability can be written as:

$$P_{\text{inf}}^{j} = 1 - e^{-r\lambda\, p_{\text{D}}(T_j, t_j)},$$

where r represents the dose-response parameter, λ the Poisson mean parameter for the bradyzoite dose, and $p_{\text{D}}(T_j, t_j)$ is the single-bradyzoite survival probability after being heated for a time t_j at fixed temperature T_j. The probability of inactivation is $p_{\text{inact,D}} = 1 - p_{\text{D}}(T_j, t_j)$, and we propose a logistic regression for it of the form

$$p_{\text{inact,D}}(T_j, t_j; \boldsymbol{\beta}) = \text{logit}^{-1}(\beta_0 + \beta_T T_j + \beta_t t_j) = 1/(1 + e^{-(\beta_0 + \beta_T T_j + \beta_t t_j)},$$

where $\boldsymbol{\beta} = (\beta_0, \beta_T, \beta_t)$. As the dose-response parameter r and the dose parameter λ cannot be separated in the experiment, we defined a new variable $\gamma = r\lambda$, and the probability of infection becomes :

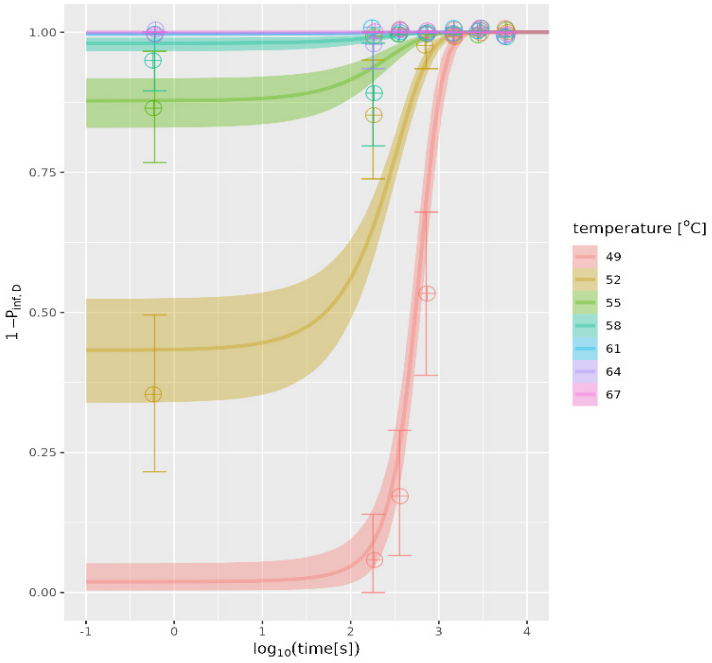
$$P_{\text{inf}}^{j} = 1 - e^{-\gamma\left(1 - p_{\text{inact,D}}(T_j, t_j; \boldsymbol{\beta})\right)}.$$

The number of infected mice k_j are assumed to be generated following a binomial process with the total number of mice n_j as follows:

$$k_j \sim Bin\left(P_{\text{inf,D}}^{j}, n_j\right).$$

By means of Markov chain Monte Carlo simulations we estimated the posterior distributions of parameters γ, β_0, β_T and β_t to the data from the experiments by Dubey et al., (1990). The Montecarlo simulations have been performed in R (R Core Team, 2024) by using the statistical Bayesian sampler JAGS (Plummer, 2003). Results of the model fit are visualised in Figure 4.

Figure 4 Probability of mice not becoming infected $(1 - P_{inf,D})$ *after being fed with infected portions of meat. The infected portions of meat have been submitted to various temperatures for the indicated time durations. Each coloured line and ribbon shows the model median and 95% uncertainty band prediction for the given fixed temperatures (indicated by colour). The mean and 95% confidence intervals from the data by Dubey et al. (1990) are indicated by circle crosses and error bars, respectively.*



The measurements by Dubey et al. are carried out using fixed temperatures and varying durations, so the model is only predicting the

probability for the simple case when $T(t) = T_j$. The calculation for varying temperatures is more complex and is relegated to Appendix B.1. To calculate the total inactivation in a meat product, we calculated p_{inact} at a hundred small volume layers and integrated them across the meat product volume V to calculate the total weighted average of p_{inact}:

$$p_{\text{inact,TOTAL}} = \frac{\int_V p_{inact}(T(V), t)dV}{\int_V dV}$$

Using the equation above, we have calculated a table containing $p_{\text{inact,TOTAL}}$ for meat products involving various shapes, sizes, cooking times, and cooking temperatures for use in the model.

3.7 Consumption data and consumer habits

3.7.1 Background

Consumer surveys are often carried out to monitor dietary intake, and these data are not specifically designed to capture behaviours associated with the risk of foodborne infections. In the One health EJP Joint Research project Toxosources we collected exposure data suitable for quantitative microbial risk assessment (QMRA) purposes from nine European countries in a harmonised way. In particular, the design of the survey enabled quantifying variation across consumers, correlations in behaviour, and uncertainty quantification. Questions were tailored to capture behaviours relevant to *T. gondii* infections. In addition to consumption frequencies and portion sizes, information on specific food handling and preparation habits that can influence the risk of *T. gondii* infection was collected. Respondents were also asked about purchases of ready-to-eat products, purchases from small-scale producers, storage conditions, and washing.

3.7.2 Survey development

Based on previous experience with a questionnaire to collect data for QMRA purposes (Chardon & Swart, 2016), a questionnaire was developed by scientists from nine European countries in collaboration with a market research agency (GfK). The questionnaire comprising 34 multiple choice questions was translated into nine languages and distributed by GfK to consumer panels in the Czech Republic, Denmark, France, Germany, the Netherlands, Norway, Poland, Portugal, and Spain. The targeted number of respondents per country was 2000, except for Denmark and Norway, which targeted 3000 respondents each. The sample was drawn representative of region (classification in supplement) and education level (low, medium, high according to classifications in supplement). Respondents completed the questionnaire between April 23 and May 17, 2021. Respondents answered questions on the frequency of consumption and portion sizes for a range of meat products (including country-specific specialties) and raw vegetables, on specific risk behaviour such as tasting raw meat and drinking unpasteurised milk, on heating preferences, storage conditions, and washing of vegetables, and on buying organic meat and ready-to-eat vegetables. To avoid the confounding effect of the COVID-19 pandemic, respondents were asked to supply answers regarding their pre-pandemic habits. For the sake of comparability with other data, the selection of product categories was based on the EFSA Foodex2 database. This

database offers a standardised classification of foodstuffs, at varying levels of detail. The baseline level used was seven, which is the most detailed level. However, products that are always cooked before consumption were considered not to present a risk of *T. gondii* infection and were therefore excluded from the questionnaire. Moreover, some categories were split by animal species (e.g. fresh meat cuts), whereas other categories were merged (e.g. sprouts and shoots) as they were considered similar in terms of risk of *T. gondii* contamination and consumption style. Both general and country-specific examples were provided for each category. In addition, for each country we asked questions on country-specific meat products that were expected to pose a high risk.

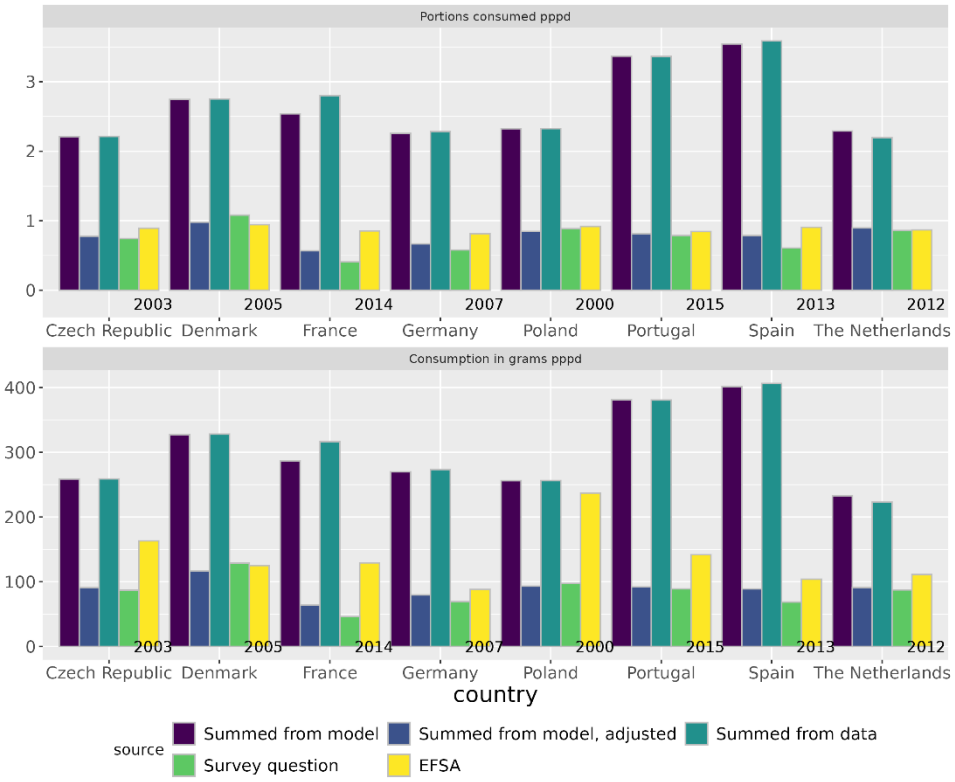
Following the survey, the data were weighted by age, gender, and education level based on Eurostat data. Raw data, including weights, were delivered by GfK to RIVM in an SPSS file. Data were imported into R for visualisation and data analysis. Bayesian statistics were employed to derive probability distributions for each question pertaining to consumption frequencies and portion sizes, to facilitate use of this information in the QMRA. The Bayesian analysis was performed by using the statistical sampler Stan (Stan Development Team, 2024) for R (R Core Team, 2024).

The detailed results of this study will be reported (Opsteegh et al., in preparation). In this report, we will highlight some specific results. Appendix C contains several tables with an overview of all information present in the survey.

3.7.3 Consumption adjustment

We compared the answer to the question on total meat consumption (C05) to the sum of the answers to all the questions on specific meat products (C02; taking the midpoints of the intervals as defined in Opsteegh et al. (in preparation). It turned out that the summed consumption frequencies far exceeded the total reported meat consumption from question C05. Evidently, respondents failed to keep a reasonable total consumption tally while answering the C02 questions, resulting in unrealistically high total consumptions. In order to obtain reasonable consumption frequencies, we applied an algorithm that iteratively decreases the consumption frequency answers of random products for each individual, until the summed frequencies do no longer exceed the total meat consumption from question C05. Figure 5 shows the effect of this adjustment. Overall, adjusted summed frequencies match the total meat consumption of C05 very well, and furthermore, compare well to independent consumption data from EFSA (European Food Safety Authority, 2011). The results presented here are all based on these adjusted responses.

Figure 5 Consumption per country in portions (top) and in grams (bottom) per person per day. The yellow bars are independent data from the EFSA Comprehensive European Food Consumption Database (European Food Safety Authority, 2011). The most recent year for which data was available is indicated above each country name. The 'Summed products' columns are summed consumption frequencies from individual meat products. The 'Survey question' column indicates the answer to the general question on total meat consumption. The columns containing 'adjusted' are the resulting frequencies following application of the adjustment algorithm.



3.7.4 Selected results and validation of the survey

Table 5 below lists some results for selected products, comparing the consumed quantities per person per year from the earlier QMRA studies, the most recent VCP Dutch food consumption survey and the Toxosources survey. For filet americain, the Toxosources survey is close to the VCP 2019-2021. The other products are consumed more frequently according to our survey than according to the other surveys.

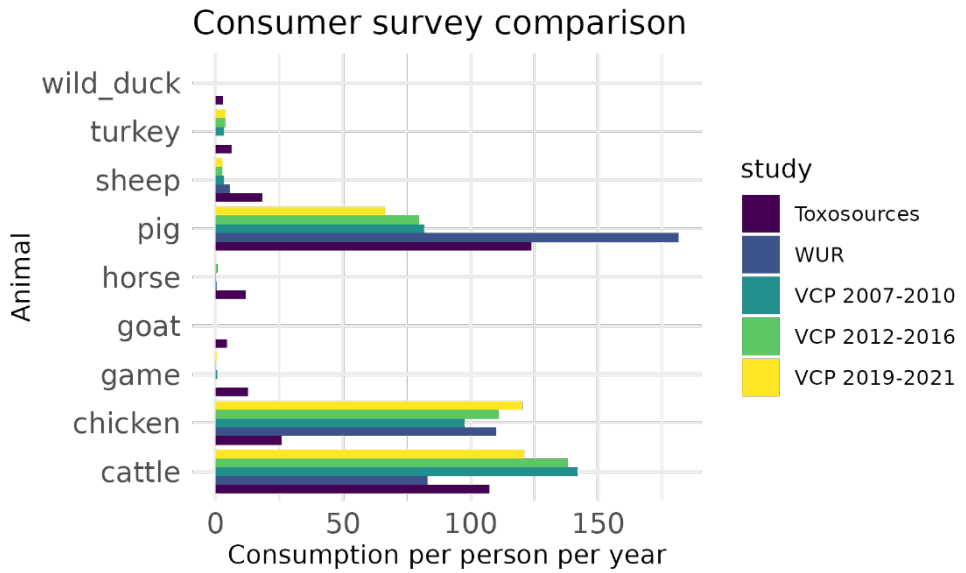
Table 5 Comparison of consumption frequencies for selected products, expressed in consumptions per person per year.

	2011 Model (VCP 1998)	**2020 Model (VCP 2007-2010)**	**VCP 2019-2021**	**Toxosources Survey[1]**
	Cons. pppy	Cons. pppy	Cons. pppy	Cons. pppy
Filet Americain	6.6	19	11	35 (consumers) 13 (all)
Mutton	0.76	0.14	0.9	21 (consumers) 10 (all)
Lamb	1.8	2.8		
Pork		82	66	213 (consumers) 123 (all)
Steak Tartare	6.6	4.2	2.4	4.1 (consumers) 3.4 (all)
Theeworst		0.4	0.4	6.1 (all)

[1] Consumers (those who eat the product sometimes) were distinguished from non-consumers (those who never eat the product). The consumption frequencies are reported for consumers only, and for the entire population (marked 'all'). Comparison to the other surveys should be based on the 'all' frequency.

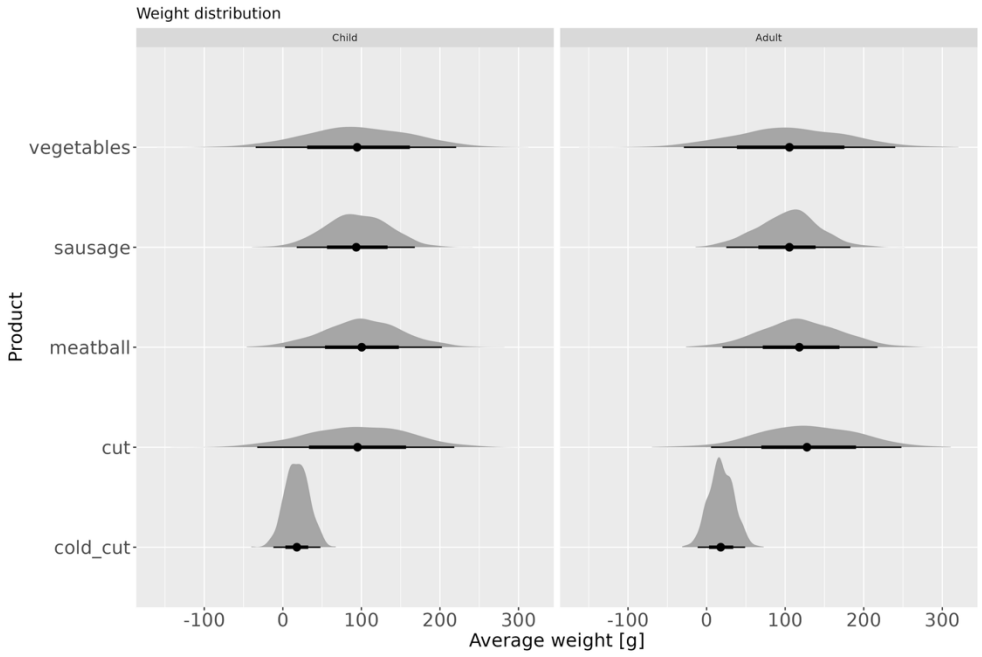
To further assess the validity of our survey and modelled outcomes, we compared the outcome at the level of the food producing animal to the Dutch food consumption survey (VCP), (van Rossum et al., 2023) and the estimates by Wageningen University (Dagevos et al., 2024).
As Figure 6 shows, in order of magnitude, our survey compares well to the VCP studies. For pig products, the WUR estimate is very far off from the other surveys. This is likely due to the different methodology employed – the estimate is based on data on slaughtered animals, combined with estimates of waste, whereas the other studies directly query consumers.

Figure 6 Comparison of consumption (portions per person per year) at the animal level between several surveys.



Apart from consumption frequencies, portion sizes are also important in the QMRA model, as they directly impact the potential number of bradyzoites in the product. As seen in Figure 7, the mean portion size of most products is around 100 g for children, and around 120 g for meatballs and meat cuts for adults. However, the spread across individuals is considerable, portion sizes of up to 150 g are not uncommon. Cold cuts (i.e. products such as sliced ham that are eaten on a sandwich for example) have a much lower portion size at about 18 g.

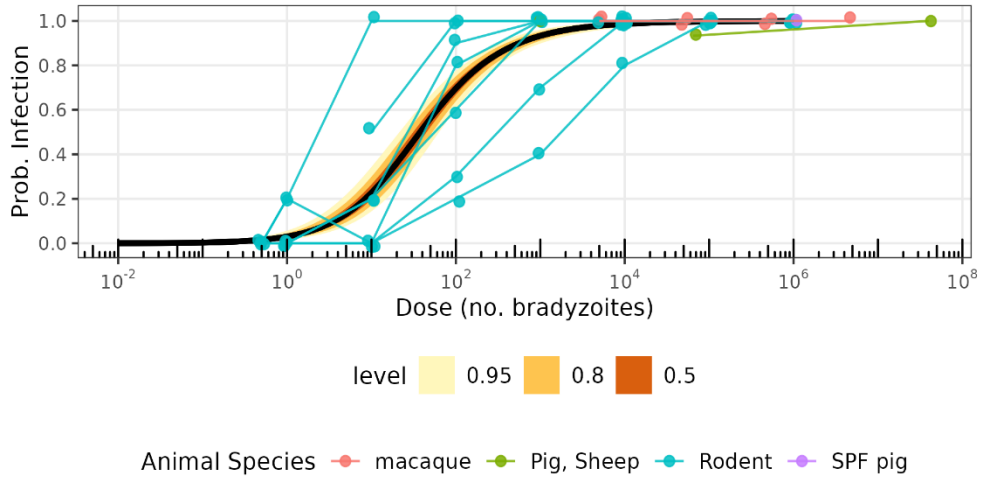
Figure 7 Portion size (weight in grams) distributions of several products, split across child (left panel) and adult (right panel). The distribution indicates the spread across consumers. The dot is the median, the thick and narrow horizontal lines cover the 50% and 95% variability interval.



3.8 Dose response

To calculate the probability of human *T. gondii* infection, a human dose-response relation upon bradyzoite ingestion is needed. Explicit dose-response relation data are available for various animals, as shown in Figure 8, but not for humans. The most relevant information comes from rodents as intermediate hosts (Dubey, 1997a, 1998), and their dose response seems similar. At the moment, for lack of better knowledge and given that humans are intermediate hosts, we assumed that the human dose-response relation is no different from that known for other intermediate mammalian hosts, i.e. mice and rats. To establish one dose-response relation on the basis of the data from several intermediate host species from various studies, we used a Bayesian framework. The Markov chain Monte-Carlo simulations of the model were carried out using the software JAGS (Plummer, 2003) version 4.3. With a Poisson distributed dose with a mean value as indicated by the measured dilution in the experiments, we assumed a Beta-Binomial probability distribution for infection given the drawn dose of bradyzoites. Figure 8 shows that, for ingestion of bradyzoites, the dose that results in a 50% probability of infection (ID50) amounts to about 40 bradyzoites (15−65, 95% confidence level). This is significantly more infectious than the dose response used in the previous QMRA models, which used an ID50 of 450 bradyzoites.

Figure 8 Dose-response relation for T. gondii infection by ingesting bradyzoites. The solid black line indicates the median dose-response relation estimated. The ribbon area indicates 95% of the most probable estimates of infection probability. The coloured dots and lines show dose-response relations observed in various mammalian intermediate hosts. Each colour represents a different animal type, as indicated in the legend, and lines join measurements from a same study.



3.9 Results at population level

The final output of the model is the age at first infection (if any) of a large number of simulated individuals. We refer to this population as the (simulated) cohort. However, a more useful outcome is the number of infections in the Dutch population. Using information on the number of individuals per age in the Netherlands, i.e. the population pyramid, we can also calculate from the cohort the infections in the Dutch population. Start with n^{cohort}, which is the total number of people simulated, for age_{max} years of age. Results will be presented by product, or animal species, and we add an index k to reference the specific product or animal. The number of times product k was consumed in the cohort at age a is written as $n_{k,a}^{\text{cohort}}$. Of those products, $m_{k,a}^{\text{cohort}}$ caused a first infection in the cohort. This means that the probability of infection from product k at age a is given by

$$p_{k,a}^{\text{cohort}} = \frac{m_{k,a}^{\text{cohort}}}{n_{k,a}^{\text{cohort}}}.$$

This probability also holds in the general population, where we have n_a^{pop} persons of age a. From the consumer survey, we know the frequency $f_{k,a}^{cohort}$ of consuming each product, and by assuming that this is representative of the frequency $f_{k,a}$ in the total population, we can calculate the number of products of type k consumed at age a in the general population as:

$$n_{k,a}^{\text{pop}} = f_{k,a} n_a^{\text{pop}}.$$

The number of persons infected in the general population then becomes:

$$m_{k,a}^{\text{pop}} = n_{k,a}^{\text{pop}} p_{k,a}^{\text{cohort}}.$$

Note that we differentiate between probability of infection as defined above, which yields the probability given consumption of the product, and risk, which also includes the frequency of consumption. On the one hand, there is the individual infection probability, which is specific to individuals who consume a specific product k. On the other hand, there is also the risk at population level, which is the probability that a random person taken from the population at age a is infected by a specific product k. In this formulation, an uninfected person could also be uninfected because they do not consume that specific product *k*. The risk at population level is given by:

$$r_{k,a}^{\text{pop}} = f_{k,a} p_{k,a}^{\text{cohort}}.$$

4 Results

The main results, expressed as cases per year in the Dutch population, and percentage attribution, are shown in Table 6. Beef remains the most important animal source of infection, as it was in the previous QMRA studies. The second-most important animal source in the Toxosources QMRA is sheep. This animal made hardly any contribution in the 2020 model but was also high in the 2011 model. Probably, this is an effect of the high consumption we find (in contrast to earlier studies), combined with a higher prevalence estimate. The role of pork as a transmission route is estimated at about 12%, very close to earlier results. The estimate for the number of people infected in the population is a little higher than estimated in the 2020 model.

Table 6 Numbers of cases and attribution percentages for the various QMRA models.

Product	2011 QMRA model Cases per year	Attribution	2020 QMRA model Cases per year	Attribution	Toxosources QMRA Cases per year	Attribution
Beef	1,904,204	67.6%	122,941	84%	80,815	46%
Veal			24	0.01%		
Mixed	270,194	7.1%	139	0.1%	-	-
Pork	427,661	11.2%	17,513	12%	21,302	12%
Lamb	533,878	14.0%	245	0.2%	18,955	11%
Mutton			6,902	3.7%	34,154	19%
Total	3,804,981		147,765		176,712	

The cumulative number of infections by age is shown in Figure 9. Infection is seen to rise steadily up to about 75% at age 80. This is close to the seroprevalence estimates reported in (van den Berg et al., 2023), which peaks at approximately the same level. However, it must be kept in mind that the current risk assessment only covers meatborne infections, while the serological estimates capture all infections, including those via vegetables and soil. Therefore, our QMRA is likely to overestimate the total number of infections.

In Figure 10 we compare consumption, probability of infection, and risk of infection for the ten most risk prone products. Note that the 'cured meat' category does not include the usual pork hams, rather it contains cured meat of sheep, goat, or game. These are rarely eaten, but have a high probability of infection. However, the most important factor in this high infectivity is the relatively low efficiency of inactivation due to salting. The conflicting data found in the literature on salting efficiency is a topic of ongoing investigation. In 2025 we will extend the current QMRA with a new salting model, which is based on new in-house mice bioassays and cell culture of salting experiments of naturally infected sheep (Opsteegh et al., 2024). As expected, steak tartare, metworst, rosbief, filet americain, theeworst and ossenworst all pose a high risk since these products are not heated. These products are salted, and as stated before, there is some uncertainty as to the actual efficiency of the salting process. In terms of probability of infection, fresh meats score high, mainly because of the high consumption. This category includes

multiple products made from various animals, and undercooking is the main risk factor. The fact that the two highest ranking products in probability of infection per person per year are rosbief and filet americain confirms expectations.
In Figure 11, we visualise Figure 10 in a different way, highlighting how some products (or animals) are more important at the individual level, while others are more important at the population level.

Figure 9 The cumulative number of infections by age, coloured by the infecting product.

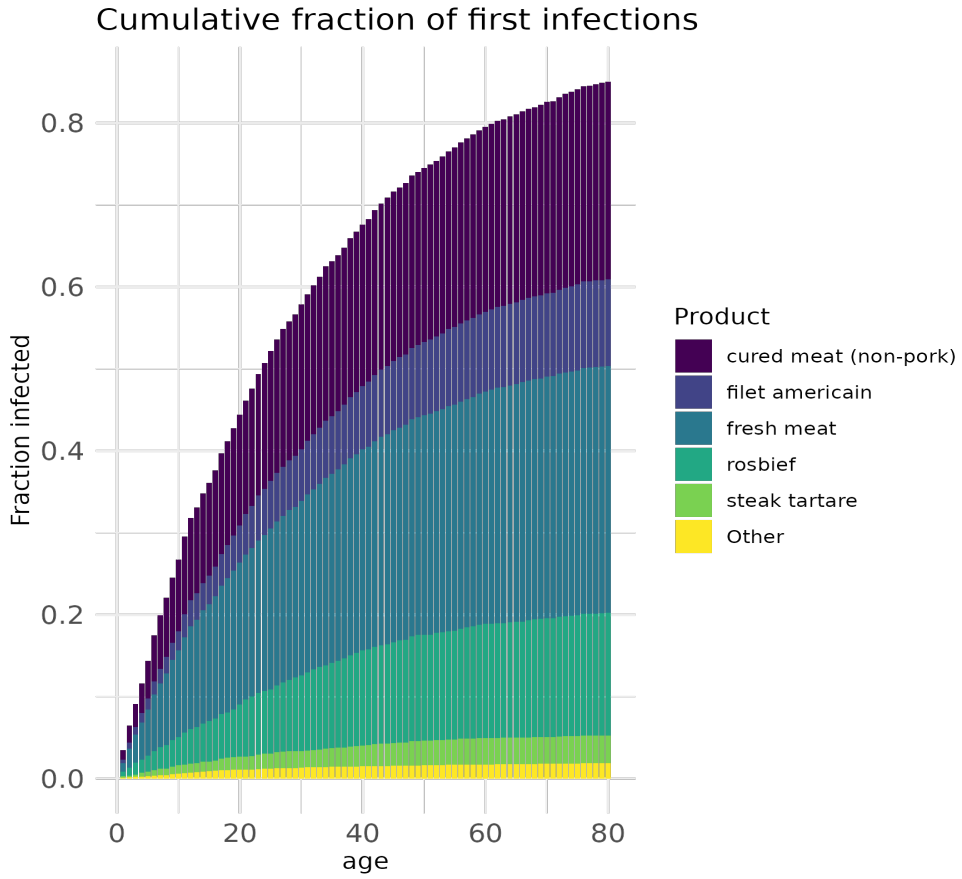


Figure 10 Risk measures for the ten products with the highest risk of infection for T. gondii. From left to right: number of consumptions per person per year, the probability of infection for a random person per year, and the risk of infection per 10,000 products.

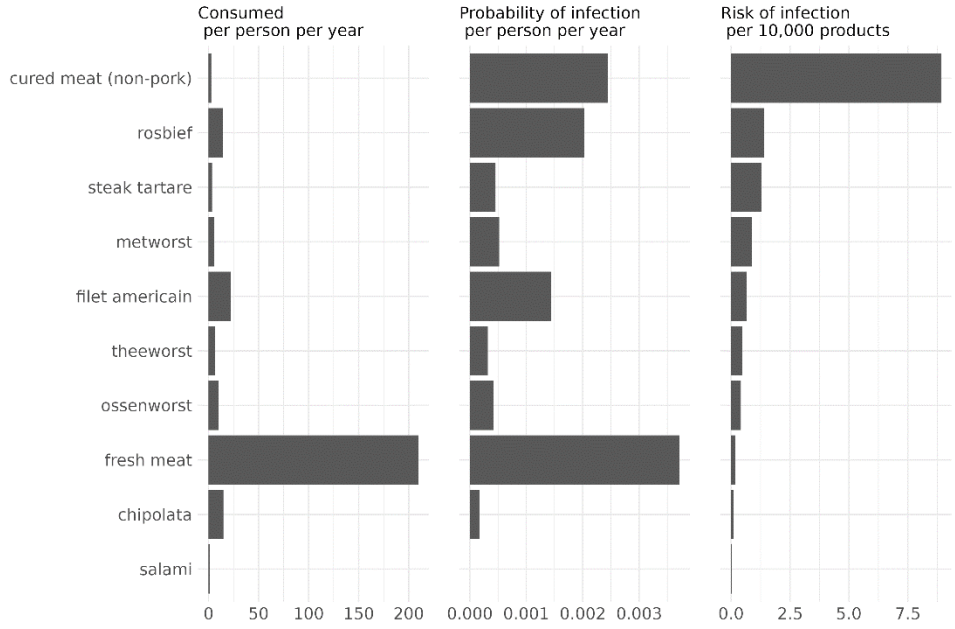
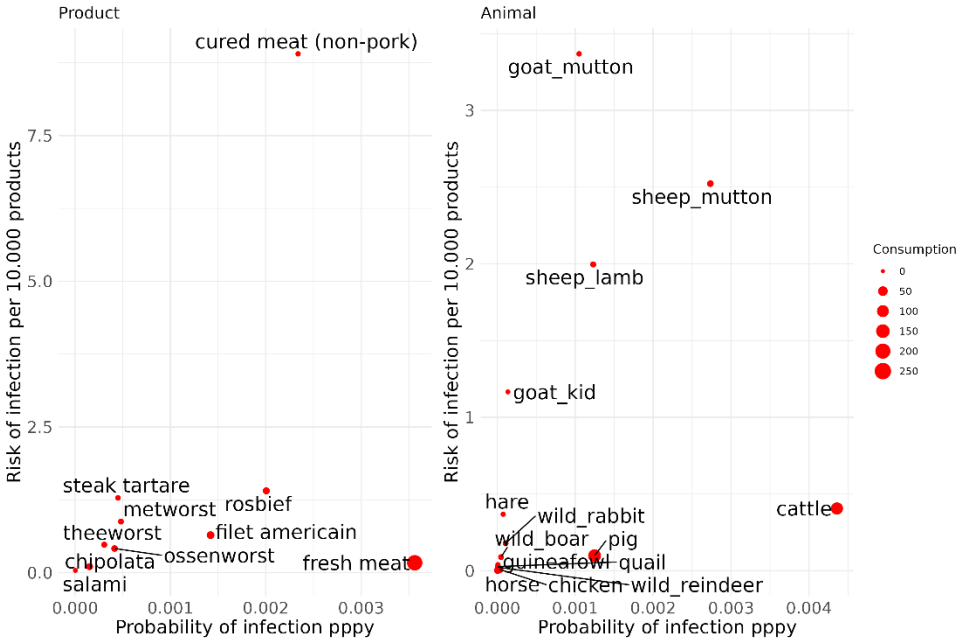


Figure 11 Relation between risk at the individual level and risk at the population level differentiated by consumed product (left) and animal source (right). On the x-axis, the risk at the population level is shown (i.e. probability of infection per year per person) and on the y-axis, the probability of infection per 10,000 products is shown.



5 Discussion

T. gondii is an important foodborne infection with a high disease burden. People can become infected through various routes, and source attribution studies are needed to develop effective prevention strategies. Since the acute stage of infection often passes unnoticed and the infection is likely to persist lifelong, it is difficult to study the sources of infection using classical methods. Moreover, since possibilities to isolate and genotype the parasite from patients and potential sources of infection are limited, molecular methods have limited use in source attribution. To fill this knowledge gap, at RIVM, we have focused on QMRA modelling for *T. gondii* source attribution. The first model was published in 2011 and updated in 2020. In this report, we present further updates of, and improvements to the previous QMRA models for meatborne *T. gondii* infections. The work on the QMRA model was performed in the OHEJP project 'Toxosources' in a European consortium involving nine countries. Moreover, within the Toxosources project, also routes of exposure to oocysts were added to the QMRA model. Here, however, we focus on the results regarding meatborne infections in the Netherlands, as this allows us to compare outcomes to previous results.

The adjustments to the previous models do not only consist of updates of input data, but also of conceptual improvements in the risk assessment model itself, as well as in the *T. gondii* inactivation models for salting, heating and freezing. Re-infections in the model are no longer corrected by simply taking into account previous immunity. Instead, individuals are now simulated throughout their lives, and only first infections are used to calculate model outputs. Previously, a direct link between processing and probability of infection in the mice was assumed in the inactivation models. This has now been corrected by directly modelling the effect of processing on the survival of viable bradyzoites. Moreover, for product heating, a spatial temperature profile was added to take into account the time and temperature at different locations in a meat product.

For most steps in the QMRA model, input data have been updated. First, the prevalence in the various animal species has been updated based on a systematic review and subsequent modelling of age-dependent prevalence in Europe (Damek, Swart, et al., 2023). This means that more and newer data are taken into account. However, the data are also less specific, as estimates for a vast territory as 'West Europe' were used, and these estimates are based on data from many different countries. All prevalence estimates were higher than the estimates based on Dutch data used in the 2011 and 2020 QMRA models. However, the parasite loads for infected animals were lowered, as now the probability that a portion does not harbour any parasites is included and input data for parasite concentration are differentiated by species and type of tissue based on literature data.

Also, product parameters have been reconsidered, collecting data from various sources and in collaboration with experts within the Toxosources consortium. Although we have done our best to get a complete picture

and have collected the data in a database that we will make publicly available, we are also aware that it is difficult to capture all possible product variations. The information available on the packaging does not provide all relevant details, and further details on industrial processing are not publicly available. For future work, a common database of product properties (and consumer behaviour) to be used in QMRA studies would be highly desirable.

The effect of freezing was simplified in comparison to previous models where an inactivation model was used. In contrast to previous models, it is now assumed that all freezing of meat is performed at a time and temperature combination that is sufficient to inactivate all *T. gondii*, which is the case in practice. Not only were the models for salting and heating updated, but also, more data was collected from the literature (on salting) or additional datapoints were used (on heating).

Particularly for salting, data from the literature is variable, and the effect on *T. gondii* viability involves uncertainty. In the future, we will add data from our own experiments (Opsteegh et al., 2024) using an in vitro assay and focus on the salting procedure as it was performed for filet americain.

To collect harmonised data on food consumption, handling and preparation in nine European countries, a survey comprising questions relevant to QMRA was performed. In the previous model, the data from the Dutch Food consumer survey (VCP) was used. One important difference is that in the VCP, data are collected by means of a two-day recall of food consumption. In our survey, we ask participants directly to indicate in general how often they consume a certain product . Also, in our questionnaire, within a product we differentiate between, for example, preparation styles as they influence the risk of *T. gondii* infection from a product. This has provided us with more relevant data, although evaluation of the answers, also made clear that these questions are more difficult to answer consistently. For example, the total consumption of meat was much lower when asked directly in comparison to the estimate based on consumption of all specific meat products combined. For this reason, we have adjusted the answers given by the respondents, artificially lowering their consumption frequency until their total consumption across all products matched the self-reported total consumption in general.
Even after this step, which results in good overall agreement with other questionnaires, we find a much higher consumption of lamb and goat than reported in the VCP. It is not yet clear what the reason for this phenomenon is. In 2025 an internal RIVM project will start ('BESMEERD') that aims to harmonise various sources of food consumption data. This research may shed some light on the issue.

As we wanted to perform source attribution based on infection, rather than at the exposure level, a dose-response model was implemented to estimate the probability of human infection. We have added further data compared to the previous models, and specifically searched for literature reporting infection experiments in non-rodent mammals. Still, most data with a good range of different doses are available from experiments in rodents.

This additional data has resulted in a marked increase in predicted infectivity. Whereas previously the dose that infects 50% of a population amounted to about 450 bradyzoites, this dose is now estimated at 40. Although this implies a large uncertainty in the number of infections, it is not likely this will impact the attribution greatly, since attribution is a relative measure within the group of infected persons. The dose-response relation will likely remain a source of uncertainty, as human infection studies are unethical, and there are rarely any outbreaks that can act as natural experiments (when outbreaks do occur, the dose received is unknown).

The results for the QMRA model are obtained by simulating a cohort of 2500 individuals for each year of age from 1 to 80. The distribution of age groups in the Dutch population is taken into account to obtain estimates that are more representative for the population. However, as consumption data were only obtained separately for those aged under 18 and those over 18 years, this is the only difference in consumption habits that is taken into account. Also, in our model, individuals aged 80 have been exposed according to our fixed data for the past 80 years, to name but one example. In reality, however, infection risks have changed over time, for example, with increased indoor livestock housing and the availability of freezers for meat storage at home. These limitations in exposure by age and over time were also present in the previous models and in QMRA models in general.

The results are presented in two ways: we have calculated the number of infections per species / product at the population level, and for these same categories, the risk per portion is also presented, as this is more relevant at the consumer level. The total number of estimated infections is reasonably in line with the previous estimate from the 2020 QMRA model (ranging from 150,000 to 175,000 infections per year). As the QMRA model is now performed by simulating infections in a cohort, the result, presented as the infected fraction by age, can easily be compared to the seroprevalence data available from the national surveys. In the latest survey for 2016-2017, the overall seroprevalence was estimated at 29.9%, with a seroprevalence of over 80% in the highest age categories (van den Berg et al., 2023). The prevalence reached in those highest age groups is in line with the results from the QMRA. However, the shape of the curve is different. The steepest increase in seroprevalence is observed in people between 60 and 70 years of age, but in the QMRA, the number of infections per year is highest in the lower age groups, as changes there are mainly due to the fact that the number of individuals available for a first infection decreases by age. It is also important to note that while only meatborne infections are considered in the QMRA, oocyst infections through exposure to soil or consumption of contaminated products (e.g. fresh produce, shellfish) are also considered an important risk of infection. Therefore, the QMRA probably overestimates the number of infections, as a fraction of the infections underlying the seroprevalence is derived via other routes.

Looking at the outputs at species and product level, the importance of beef and filet americain is consistent between this QMRA model and the two previous ones, even though the attribution to beef had decreased to 46%. At product level, rosbief and steak tartare present a higher risk

than filet americain and, taking consumption into account, at population level too, rosbief is now estimated to be slightly more important than filet americain. For meat products such as filet americain and rosbief, which are processed and bought ready to eat, it is important to take into account that there is uncertainty in the processing parameters and the effect of salting on *T. gondii* viability.

One important difference compared to the previous QMRA models is the increased importance of fresh meat products. Consumption of fresh meat products was and still is high, but the model for the effect of heating on *T. gondii* viability has been modified, taking into account that heat is not equally distributed over the product and that fresh meat products are now estimated to be the most important source of infections at population level. Of course, for fresh meat products, the risk for individual consumers depends heavily on preparation habits, and infection can be prevented by freezing or properly heating these products.

Another difference compared to the previous QMRA models is the increased number of predicted infections due to lamb and goat. This is driven by our higher estimate of lamb and goat consumption as compared to the Dutch food consumption surveys (VCP). The origin of this discrepancy is as yet unclear.

In presenting the results of this QMRA, we have tried to highlight the difference between the risk of a product at consumer level (risk per portion) and at population level (infections in the population). Some products, such as steak tartare and lamb and mutton, have a high risk per portion, but are not consumed that often and do not contribute that significantly to the infections in the population. Whereas, for example, fresh meats do not carry a high risk per portion, they are calculated to be an important risk at population level. These differences have implications for infection prevention. The products that present the highest risks at consumer level need to be specifically addressed in food safety information for risk groups, such as pregnant women and severely immunocompromised patients. However, for prevention strategies aimed at reducing *T. gondii* infections in the population by targeting the sources of infection, it is likely more effective to focus on product groups or animal groups that pose higher risks at population level.

In conclusion, the work presented here has substantially improved the QMRA model to estimate the meatborne *T. gondii* infections in the Netherlands. The conceptual model has been improved and updated with new data. Although attributions per product have shifted, beef and raw beef products are still estimated to be the most important sources of infection in the Dutch population. However, aside from the unavoidable uncertainty in the dose-response, the effect of salting, meat processing in industry, consumption and preparation by consumers, which all strongly influence model outcomes, hold a certain degree of uncertainty as well. Future work will focus on these uncertainties. Inactivation data for salting, as performed in filet americain production, will be added to the QMRA model. In a 2025 project, consumption data from different sources will be collected and compared.

The forthcoming model at the EU level, also including vegetable and soil routes of transmission, will be a more comprehensive tool that offers further validation opportunities by relying upon epidemiological data of other countries, gives us more insight into the accuracy of the predictions, and provides a global picture of the number of infections at European level.

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Food products glossary

Filet americain	A spread of minced raw beef and sauce (mayonnaise and spices)
Paardenrookvlees	Salted and smoked thin slices of horse meat
Ossenworst	A spiced and salted raw beef sausage
Theeworst	A spreadable raw pork sausage
Rosbief	Beef, roasted in large chunks with interior still red but the crust well done. Also sold in thin slices to use as bread topping.

Appendix A Salting Model

We will build the inactivation model by making the data generating process explicit. In our case, the data is: $\boldsymbol{x}^j$ (experimental settings for experiment j), n^j (number of mice bioassayed at experiment j) and k^j (number of positive mice at experiment j). The rate of bradyzoite occurrence λ^k is modelled as varying across studies k, since a single study typically feeds mice from a single stock.
This generates a dose for mouse i $(1 \leq i \leq n)$ via:

$$D_i \sim \text{Poisson}(\lambda^k)$$

We model organism survival probabilities of salting using a logistic regression as follows:

$$p(\boldsymbol{x}^j; \boldsymbol{\beta}) = \text{logit}^{-1}(\boldsymbol{x}^j \cdot \boldsymbol{\beta}).$$

This lowers the dose after salting to:

$$D^j_{i,\,\text{salted}} \sim \text{Binomial}(p(\boldsymbol{x}^j; \boldsymbol{\beta}), D_i).$$

In the next stage, the organism must survive the host's defences to initiate infection. Using the single hit probability of infection r, the number of surviving organisms is:

$$D^j_{i,\,\text{surv}} \sim \text{Binomial}\left(r, D^j_{i,\,\text{salted}}\right).$$

The probability of infection for mouse i is:

$$P_{i,\,\text{inf}} = 1 - P\left(D^j_{i,\,\text{surv}} = 0\right).$$

and the total number of infected mice is:

$$k_i \sim \text{Binomial}\left(n_i, P_{i,\,\text{inf}}\right).$$

This approach can be simplified before implementation into the QMRA model. Indeed, the binomial-Poisson compound distributions yield a familiar exponential dose-response type relation:

$$P_{i,\,\text{inf}} = 1 - e^{-r\,p(\boldsymbol{x}^j;\boldsymbol{\beta})\lambda^k},$$

which can also be seen to be independent of i. The binomial distribution for the number of infected mice simplifies to:

$$k_i \sim \text{Binomial}\left(n_i, 1 - e^{-r\,p(\boldsymbol{x}^j;\boldsymbol{\beta})\lambda^k}\right).$$

Note that r and λ^k are not identifiable at this point, and we estimate them jointly as $\gamma_k = r\lambda^k$.

Appendix B Heating model

The temperature profile can be modelled by using the standard formula for heat diffusion:

$$\frac{\partial T}{\partial t} = \alpha \nabla^2 T$$

where T is temperature, t is time, ∇^2 is the second derivative on position, and α is the thermal diffusion coefficient.
Depending on the geometry of the meat product being cooked, solutions for this equation can be calculated, and we considered meatballs (sphere), sausages (cylinder), and burgers or steaks (slab).
Under all geometries, we consider R to be the distance from the centre/core ($r = 0$) of the meat product to its surface. We assumed that across the whole meat product, the initial temperature is uniform $T(r) = T_0, 0 \leq r \leq R$, and the boundary condition $T(R) = T_{\text{cooking}}$, i.e. the temperature of the outermost layer is the same as the temperature of the heating medium (oven, water, oil, etc.), but maximised at 100 °C since the water present in the meat cannot reach higher temperatures.
The solutions for expressing temperature in terms of position and time for each geometry under the assumed boundary conditions are given in Equations (1-3) below

For a sphere of radius R (e.g. meatballs),

$$\frac{T(r,t) - T_{\text{cooking}}}{T_0 - T_{\text{cooking}}} = \sum_{n=1}^{\infty} (-1)^{n+1} 2 e^{-\lambda_n^2 \alpha t} \frac{\sin(\lambda_n r)}{\lambda_n r} \qquad (1)$$

where $\lambda_n = \frac{n\pi}{R}$.

For a (infinitely) long cylinder of radius R (e.g. sausages),

$$\frac{T(r,t) - T_{\text{cooking}}}{T_0 - T_{\text{cooking}}} = \sum_{m=1}^{\infty} 2 e^{-\lambda_m^2 \alpha t} \frac{J_0(\lambda_m r)}{\lambda_m R J_1(\lambda_m R)} \qquad (2)$$

where J_n is the Bessel function of the first kind, $\lambda_m = a_m/R$, and a_m is the m^{th} point where J_0 is zero, defined by $J_0(a_m) = 0$ for $m = 0, 1, 2, \ldots$.

For an (infinitely) extended slab of half-thickness $x = R$ with position coordinate $x = 0$ starting in the center of the piece, and cooked on both sides (e.g. hamburgers),

$$\frac{T(x,t) - T_{\text{cooking}}}{T_0 - T_{\text{cooking}}} = \sum_{n=1}^{\infty} 4 e^{-\lambda_n^2 \alpha t} \frac{\sin(\lambda_n (R - x))}{n} \qquad (3)$$

where $\lambda_n = \frac{n\pi}{R^2}$.

Time- and temperature-dependent probability of inactivation

The measurements by Dubey et al. are carried out at fixed temperatures and varying durations, so the model is only predicting the probability $p_{\mathrm{inact,D}}(T_j, t_j; \boldsymbol{\beta})$ from line integrals in the $T-t$ plane that follow curves with parametrisation $\boldsymbol{r}(t) = (T(t), t)$ for the simple case when $T(t) = T_j$. We may write a line differential to relate our inactivation probability to a general probability density $f(\boldsymbol{r})$ by the path integral

$$p_{\mathrm{inact}} = \int_C f(\boldsymbol{r}) ds.$$

By definition, $dp_{\mathrm{inact,D}} = f(\boldsymbol{r}) ds$, therefore,

$$\frac{\partial p_{\mathrm{inact,D}}}{\partial t}\mathrm{dt} + \frac{\partial p_{\mathrm{inact,D}}}{\partial T}\frac{\mathrm{dT}}{\mathrm{dt}}\mathrm{dt} = \mathrm{f(T,t)}\sqrt{1+\left(\frac{dT}{dt}\right)^2}\,\mathrm{dt},$$

which yields

$$\mathrm{f}(T,t) = \frac{\frac{\partial p_{\mathrm{inact,D}}}{\partial t} + \frac{\partial p_{\mathrm{inact,D}}}{\partial T}\frac{\mathrm{dT}}{\mathrm{dt}}}{\sqrt{1+\left(\frac{dT}{dt}\right)^2}}.$$

Using the inverse logistic function arithmetic properties, we found that the full probability density of inactivation for a bradyzoite is:

$$f(T,t) = \frac{p_{\mathrm{inact,D}}(T,t;\boldsymbol{\beta})\left(1-p_{\mathrm{inact,D}}(T,t;\boldsymbol{\beta})\right)}{\sqrt{1+\left(\frac{dT}{dt}\right)^2}}\left(\beta_T \frac{dT}{dt} + \beta_t\right).$$

Given that we know, from the heating profiles, the pathway in the temperature-time plane that any point in the meat product will follow, the total probability of inactivation, p_{inact} as defined above, is then straightforward to integrate numerically. From the experiments by (Dubey et al., 1990), it is concluded that even for small time exposures at a temperature of 67 °C and over, complete inactivation is observed, i.e. $p_{\mathrm{inact}} = 1$. Also, as *T. gondii* infects warm-blooded animals, including birds, we took a temperature limit of 43 °C below which we assumed that no inactivation happens, i.e. $p_{\mathrm{inact}} = 0$.

$$p_{\mathrm{inact}}(T,t,\boldsymbol{\beta}) = \begin{cases} 0 & \text{for } T < 43 \\ \sum_i p_{\mathrm{inact,D}}(T_i,t_i,\boldsymbol{\beta})\left(1-p_{\mathrm{inact,D}}(T_i,t_i,\boldsymbol{\beta})\right)\frac{\beta_T \Delta T_i + \beta_t \Delta t_i}{\sqrt{\Delta T_i^2 + \Delta t_i^2}} & \text{for } 43 \le T \le 67 \\ 1 & \text{for } T > 67 \end{cases},$$

where i indicates each step pair $\boldsymbol{r}_i = (T_i, t_i)$ tracing the followed path on the temperature-time plane from $r_0 = (T_{0,}0)$ to $r = (T,t)$.

Appendix C Survey Tables

Table 7 Summary of queries and answer options on dietary habits, consumption frequencies, and portion sizes of selected risk food items, and specific behaviours linked to food-borne infection in the online survey.

Dietary habits, consumption frequencies and portion size

Code	*Questions*	Answer type *	Multi-choice answer options
C01	Adherence to specific diets	A	Vegetarian; vegan; pescatarian; raw food diet; kosher; halal; avoid specific products due to pregnancy; avoid specific products due to compromised immunity; avoid specific products due to allergies/intolerances; diet for other medical reasons; diet to change body condition; don't answer; no
C02	Consumption frequencies for meat and vegetable/fruit products listed in (Opsteegh, in preparation)	B.1	Daily, >3 times per wk; 2–3 times per wk; 1 time per wk or 2–4 times per mo; ≤ 1 time per mo; never
C03a	Doneness preference of fresh meat cuts, minced/ground meat products or sausages (all species)	A	Well done; medium; rare; raw
C03b	Type of dry-fermented sausages consumed	A	Dry-hard shelf stable fermented sausages; semi-dry fermented sausages; both types; don't know
C03c	Type of poultry meat consumed	A	Chicken; turkey; farmed duck; farmed goose; ratite / ostrich; other
C03d	Type of lamb, mutton or goat meat consumed	A	Sheep lamb; goat lamb; mutton (adult sheep); adult goat
C03e	Type of other livestock meat consumed	A	Donkey / mule; buffalo; farmed rabbit; farmed reindeer; other farmed species
C03f	Type of game meat consumed	A	Wild boar; rabbit, hare; reindeer; moose; other deer; mouflon; chamois; ibex; other
C03g	Type of wild birds consumed	A	Wild duck; wild goose; pheasant; quail; partridge; grouse; guineafowl; pigeon; ptarmigan; other
C04	Portion size of raw meat tasted	C	> 1 teaspoon; 1 teaspoon; 1 tablespoon; > 1 tablespoon

Code	*Questions*	Answer type *	Multi-choice answer options
C05	Consumption frequency for meat overall	B.2	≥ 3 times per day; 1-2 times per day; 2-6 times per wk; 2-4 times per mo; ≤ 1 time per mo; never
C06a	Portion size for meat balls	C	75 g; 100 g; 135 g; 190 g; 265 g; never **
C06b	Portion size for sausages	C	55 g; 100 g; 135 g; 160 g; never**
C06c	Portion size for meat cuts	C	20 g; 35 g; 75 g; 115 g; 170 g; 275 g; never**
C07	Portion size for cold cuts	C	Amount portions (approx. 10g per portion) per consumption moment; 0 if not eaten**
F01	Consumption frequency for unpeeled raw fruit	B.2	≥ 3 times per day; 1-2 times per day; 2-6 times per wk; 2-4 times per mo; ≤ 1 time per mo; never
F02	Consumption frequency for unpasteurised juice or smoothie with raw vegetables or unpeeled fruits	B.2	≥ 3 times per day; 1-2 times per day; 2-6 times per wk; 2-4 times per mo; ≤ 1 time per mo; never
F03	Consumption frequency for raw vegetable products listed in table	B.2	≥ 3 times per day; 1-2 times per day; 2-6 times per wk; 2-4 times per mo; ≤ 1 time per mo; never
F04	Portion size of raw vegetables	C	Amount portions (approx. 55 g per portion) per consumption moment; 0 if not eaten**

Consumption behaviours and preferences associated to the risk of food-borne infections

Code	*Questions*	Answer type *	Multi-choice answer options
T01	Consumption fraction for ready-to-eat raw vegetables	D	(almost) 0 in 4 times; 1 in 4 times; 2 in 4 times; 3 in 4 times; (almost) 4 in 4 times; unknown
T02	Consumption fraction raw consumed vegetables from small-scale producer, own vegetable garden, self-grown herbs or picked from the wild	D	(almost) 0 in 4 times; 1 in 4 times; 2 in 4 times; 3 in 4 times; (almost) 4 in 4 times; unknown
T03	Frequency portions frozen before consumption (all meat eaten)	D	(almost) 0 in 4 times; 1 in 4 times; 2 in 4 times; 3 in 4 times; (almost) 4 in 4 times; unknown
T04	Consumption frequency for organic or free-	D	(almost) 0 in 4 times; 1 in 4 times; 2 in 4 times; 3 in

Code	*Questions*	Answer type *	Multi-choice answer options
	range type meat (all meat eaten)		4 times; (almost) 4 in 4 times; unknown
T05	Frequency hand washing before consumption of products 1-14	D	(almost) 0 in 4 times; 1 in 4 times; 2 in 4 times; 3 in 4 times; (almost) 4 in 4 times; unknown
T06	Storage place of products listed in tables	A	Direct consumption; mostly outside fridge or freezer; mostly fridge; equally often fridge and freezer; mostly freezer; unknown; not done
T07	Cooling capacity freezer/freezing compartment used for meat storage	A	≤ -6 °C; ≤-12 °C; ≤-18 °C; temperature not shown; unknown; don’t have
T08	Frequency outdoor soil contact, e.g. garden and/or sandbox?	B.1	Daily, >3 times per wk; 2–3 times per wk; 1 time per wk or 2–4 times per mo; ≤ 1 time per mo; never

Table 8 Food categories (vegetables and meat products) queried in the online survey and respective coding

Code	Vegetable categories	Specified products and examples
C02_1	Berries and small fruits	All berries, e.g. raspberries, grapes, strawberries, blueberries, cranberries, currants, blackcurrants, blackberries, elderberries, cloudberries, lingonberries, redcurrant
C02-2	Unpeeled Pome Fruits	Apples, pears
C02_3	Flowering brassica	Broccoli, cauliflowers, romanesco
C02_4	Cucurbits	Cucumber, courgette/zucchini
C02_5	Tomatoes	
C02_6	Peppers	Sweet pepper, peppers
C02_7	Fungi	Common mushrooms, wild fungi and similar-, jew's ears, nameko, pearl oyster mushrooms, shiitake, fungi, ceps, chanterelles, horns of plenty, morels, saffron milk cap, truffles, cultivated fungi and similar, honey mushroom
C02_8	Fresh herbs and edible flowers	Dill leaves, chives, parsley, coriander leaves, basil, mints, fennel leaves, common Sorrel, wood sorrel, ramson, young pea plant, elderflower
C02_9	Leafy vegetables	Spinaches, chards, swiss chards, glassworts, purslanes, lettuces, endives, crisp lettuces, head lettuces, dandelions, escaroles, radicchio, lollo rosso, romaines, roman rocket, baby leaf, beetroot leaves
C02_10	Sprouts, shoots and similar	Sprouts (alfalfa, lentil, mungbean) and cresses, watercress
C02_11	Other leafy vegetables	Witloof, cabbages, kale, pak-choi
C02_12	Root and tuber vegetables	Carrots, radishes
C02_13	Stems/stalks	Celeries, florence fennel, leeks, spring onions, welsh onions, rhubarb
C02_14	Fermented vegetables	Sauerkraut, kimchi, pickles
C02_15	Asparagus (mini)	
C02_16	Sugarsnap peas	
C02_17	Ginger and curcuma roots	
C02_18	Salicornia	

Code	Meat categories	Specified products and examples
C02_19	Beef/veal	steaks, roasts, chops (bone-in, boneless, portioned), trimmings, fillet, entrecote, loin, cutlet, kebab, leg
C02_20	Pork	loin, chops, cutlets, steaks, fillet, trimmings, medallions, belly
C02_21	Poultry: chicken, turkey, farmed duck, farmed goose, ratitis/ostrich, etc.	breast, drumstick, wing, fillet leg, medallions, heart, kebab
C02_22	Lamb (sheep or goat)	saddle, shoulder, neck, leg, cutlet, kebab, lamb crown, fillet
C02_23	Mutton or goat	loin, chops, fillet, steak
C02_24	Horse	steak, fillet, entrecote
C02_25	Other livestock species (not wild) or unknown: farmed rabbit, reindeer, donkey, buffalo, etc.	fillet, leg etc.
C02_26	Game (wild mammals): wild deer (all types including reindeer), wild boar, moose, elk, hare, rabbit, mouflon, chamois, ibex, etc.	loin, leg, steak, entrecôte, fillet, saddle, ribs, etc.
C02_27	Wild birds: wild goose, pheasant, grouse, pigeon, partridge, quail, guineafowl, wild duck, etc	breast, fillet, whole, etc.
C02_28	Beef/veal	burgers, patties, meatballs, meatloaf
C02_29	Pork	burgers, patties, meatballs, meatloaf
C02_30	Mixed beef/pork	burgers, patties, meatballs, meatloaf
C02_31	Other: other than beef, veal, pork or beef/pork mixtures	burgers, patties, meatballs, meatloaf
C02_32	Fresh sausages (all species)	Chipolata-type sausage, Fresh bratwurst, Fresh raw sausages, Fresh spiced sausages in casing, Breakfast-type sausage
C02_33	Raw meat spread, raw meat based spreadable-textured specialties (all species	sobrassada, nduja, spreadable mett(wurst)
C02_34	Raw cured meat: pork (e.g. raw ham-like and raw bacon-like)	raw ham (e.g. parma, serrano), prosciutto, bacon, pancetta

Code	Meat categories	Specified products and examples
C02_35	Raw cured meat: beef (e.g. smoked beef)	smoked raw beef, Bresaola
C02_36	Other species or unknown: other than beef or pork	cured meat/ham from horse or game or any species other than pork or beef
C02_37	Dry fermented sausages, often smoked, salami-like (all species)	salami, chorizo, Genoa Salami, Italian dry salami hungarian dry salami (horse), sopressata, cold smoked sausage from any species
C02_38		Dried meats/jerky (all species)
C02_39		Carpaccio
C02_40		Steak tartare

Table 9 Meat specialties, specific for the Netherlands, queried in the online survey

Country	Code	Country-specific product
Netherlands	C02_55	Filet_americain
	C02_65	Paardenrookvlees
	C02_75	Ossenworst
	C02_85	Theeworst
	C02_95	Rosbief

Appendix D Consumption Model

The survey questions are multiple choice questions with *k*= 1 ,.., *K* possible answers. For each answer *k,* we choose an upper and lower bound, such that the answer corresponds to the interval $[a_k, b_k]$, as in Opsteegh et al. (in preparation). In this way, we account for the fact that the answer cannot be translated in one exact frequency but there is a range of variability around it. We set a_1 to zero and b_K to a very large number. Let Y be the random variable describing the consumption frequencies expressed in consumption moments per day. We assume $Y \sim \mathrm{Beta}(\alpha, \beta)$ with parameters α and β. Here, we use a re-parametrised version of the Beta distribution with parameter $\phi = \alpha/(\alpha + \beta)$ and parameter $\lambda = \alpha + \beta$. In this new parametrisation, ϕ can be interpreted as the mean frequency of consumption and λ as the variability (spread) around the mean.
Denote the answer of individual i by A_i, then the likelihood individual contribution is written in terms of the cumulative distribution (CDF) of the beta distribution as :

$$L_i = F_Y\big(b_{A_i}; \phi, \lambda\big) - F_Y\big(a_{A_i}; \phi, \lambda\big).$$

Let's now call n_k the number of times answer k was supplied ($n = \sum_k n_k = \sum_i A_i$), then the total log-transformed likelihood reads:

$$\mathcal{L} = \sum_{k=1}^{K} n_k \log\big(F_Y(b_k; \phi, \lambda) - F_Y(a_k; \phi, \lambda)\big).$$

D.1 Zero inflation

In many questions, an overabundance of zeros (i.e. answer "never") was noticed. In the model, as described above, "never" was implicitly assumed to mean "very rarely or never" but there were too many entries in this category to be captured by the beta distribution. Therefore, to distinguish between the true "never" (non-consumers) and "very rarely" we applied zero inflation to the model. Specifically, we introduced p_{nc}, the probability of a person being a non-consumer and we assign to "never" a probability p_{nc} of being a true non-consumer and a probability $1 - p_{\mathrm{nc}}$ to a potential consumer. The likelihood of the "never" category (*k*=1) needs to be redefined as:

$$P(Y \le y) = p_{nc} + (1 - p_{nc}) F_Y(y; \phi, \lambda)$$

This turns the log-likelihood into:

$$\mathcal{L} = n_1 \log\Big((1 - p_{nc})\big(F_Y(b_1; \phi, \lambda) - F_Y(0+; \phi, \lambda)\big) + p_{nc}\Big) + (n - n_1)\log(1 - p_{nc}) + \sum_{k=2}^{K} n_k \log\big(F_Y(b_k; \phi, \lambda) - F_Y(a_k; \phi, \lambda)\big)$$

Estimation of the parameters ϕ and λ of the beta distribution, for each combination of products (question) and country, for the total population,

and for the population differentiated into adults and children, was performed in a Bayesian setting using Hamiltonian Monte Carlo implemented in Stan (Carpenter et al., 2017) by using the R package ‘rstan’. The code is made available at https://github.com/rivm-syso/Toxosources.

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