Deliverable D-JRP-TOXOSOURCES-WP2.4

Report on relative contribution of different sources and routes of exposure by country/region

Workpackage 2 of JRP22-FBZ4.1-TOXOSOURCES

Responsible Partners:

RIVM, SLV, DTU, SSI

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D-JRP-TOXOSOURCES-WP2.4
REPORT ON RELATIVE CONTRIBUTION OF DIFFERENT SOURCES AND ROUTES OF EXPOSURE BY COUNTRY/REGION

BACKGROUND

This is a public deliverable of One Health EJP Joint Research Project:
JRP22-FBZ4.1-TOXOSOURCES – *Toxoplasma gondii* sources quantified
(https://onehealthejp.eu/jrp-toxosources/);

Work Package:
JRP-TOXOSOURCES-WP2 Multicentre quantitative microbiological risk assessment for *T. gondii* infections;
Task:
JRP-TOXOSOURCES- WP2-T1 QMRA modelling for human *T. gondii* infections

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TOXOSOURCES addresses the research question – **What are the relative contributions of the different sources of *T. gondii* infection?** – by using several multidisciplinary approaches and novel and improved methods to yield robust estimates that can inform risk management and policy makers.

TOXOSOURCES WP2 estimates the relative contribution of different sources of *T. gondii* infection by quantitative microbiological risk assessment (QMRA).
Objectives of TOXOSOURCES WP2:

- ✓ To estimate the relative contribution of food and environmental transmission routes (T1)
- ✓ To provide an overview of the prevalence in food animals and cats (T2)
- ✓ To quantify human exposure to possible sources of infection (T3)
- ✓ To provide an overview of the processing parameters for relevant meat products (T4)
- ✓ To provide an overview of prevalence and risk factors of human infection (T5)

To help achieve the goals of TOXOSOURCES WP2, within the task WP2-T1 a quantitative risk assessment was performed to identify the relative importance of different sources of human *T. gondii* infection in Europe. The task was performed successfully in collaboration by scientists from related fields from several partner institutes across Europe. The work included integrative aspects in terms of collaboration and harmonising the process, and cross-sectoral aspects in terms of collaboration within the consortium and specifically with WP3, and integrated results from different activities of the consortium. Capacity-building was also included, and both experienced and early-career scientists participated in the work.

Dissemination of the outcomes is ongoing in collaboration with TOXOSOURCES WP1 and following the FAIR principles. This Deliverable reports on the work done and highlights the key achievements of the process.

PURPOSE

The aim of the work was to estimate the relative contribution of different sources of *T. gondii* infection by quantitative micribiological risk assessment (QMRA). The work builds on previous work performed at RIVM for *T. gondii* infections in the Netherlands (Deng et al., 2021; Deng et al., 2019; Opsteegh et al., 2011).

MATERIALS AND METHODS

The QMRA model work consisted of several steps (Fig. 1), for which data were gathered and models established using coordinated collaborative approaches:

- The prevalence of *T. gondii* infection in livestock and wildlife in Europe (TOXOSOURCES WP2-T2, D-JRP-TOXOSOURCES-WP2.1) and anatomical distribution (previous work, literature, One Health EJP PhD project ToxSauQMRA which collaborates closely with TOXOSOURCES)
- Presence of *T. gondii* contamination on fresh produce (TOXOSOURCES WP3)
- Processing information for generic and country-specific meat products (TOXOSOURCES WP2-T4) and the effect of heating, freezing and salting on the viability of *T. gondii* (previous work, literature, One Health EJP PhD project ToxSauQMRA)
- Food habits and other exposure behavior in the Czech Republic, Denmark, France, Germany, the Netherlands, Norway, Poland, Portugal, and Spain (TOXOSOURCES WP2-T3, D-JRP-TOXOSOURCES-WP2.2)
- Dose-response model (previous work, literature, One Health EJP PhD project ToxSauQMRA)
- Prevalence and risk factors of human *T. gondii* infections in Europe (TOXOSOURCES WP2-T5)

This work would not have been possible without multidisciplinary international collaboration. In the following sections, we will briefly highlight the main achievements for each key step. More details are available in earlier TOXOSOURCES deliverables and publications, all of which are available (FAIR principle) via Zenodo: https://zenodo.org/search?page=1&size=20&q=keywords:%22TOXOSOURCES%22&sort=mostrecent.

The models were implemented in R version 4.2.1. All models and data will be available (FAIR-principle) at https://github.com/arno314/Toxosources and the results of the work are being written into a scientific publication (2023).

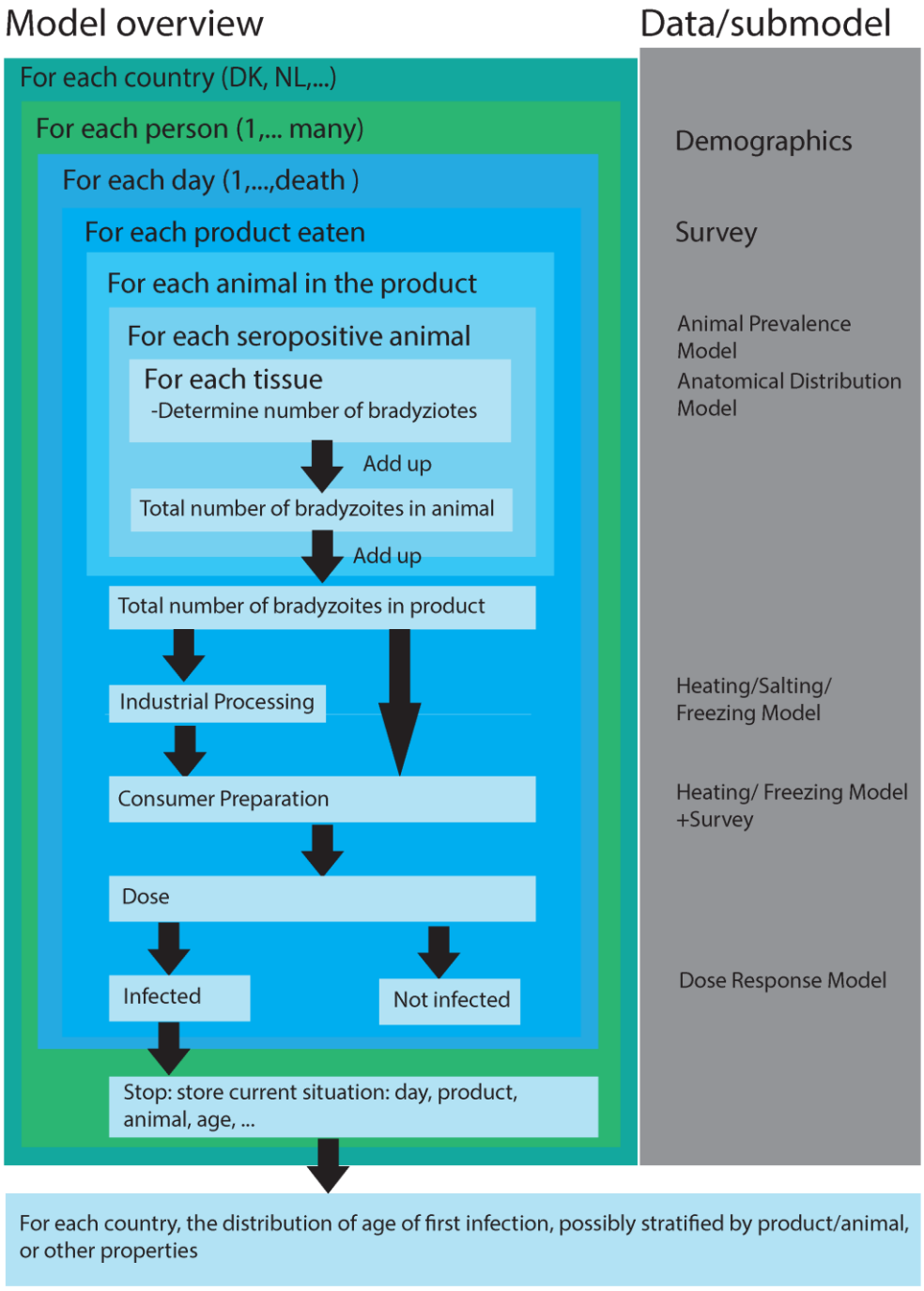

Figure 1. Structure of the QMRA model

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KEY ACHIEVEMENTS AND UNIQUE STRENGTHS OF THE TOXOSOURCES-QMRA AND DATA BEHIND IT

1. Prevalence in Animals

A systematic literature review was performed to gather information on prevalence of *T. gondii* in several animal species in Europe. Subsequently, the data were analysed using a Bayesian hierarchical model coupled with a age-dependent transmission model. Several covariates were included in the model (e.g. outdoor access, sample matrix). This sub-model enabled us to input animal prevalences for each relevant species as a starting point of the QMRA.

2. Anatomical Distribution

Once an animal has been marked as infected in the model, the question is which tissues of the animal have infective parasites, and to what degree. To answer this question data have been gathered from the scientific literature on infection levels (numbers of bradyzoites per gram) in tissues of animals. A model was developed with 'animal species', 'tissue' and 'type of dosage' as covariates. The type of dosage could be either oocysts, tissue cysts, or natural infection. Particularly this last aspect is important, since it allows us to model the expected bradyzoite load stemming from natural infection of the animal.

The model consists of two parts 1) is a tissue infected 2) if yes, with what amount of bradyzoites per gram (Figure 2). There is considerable variation between species and tissue.

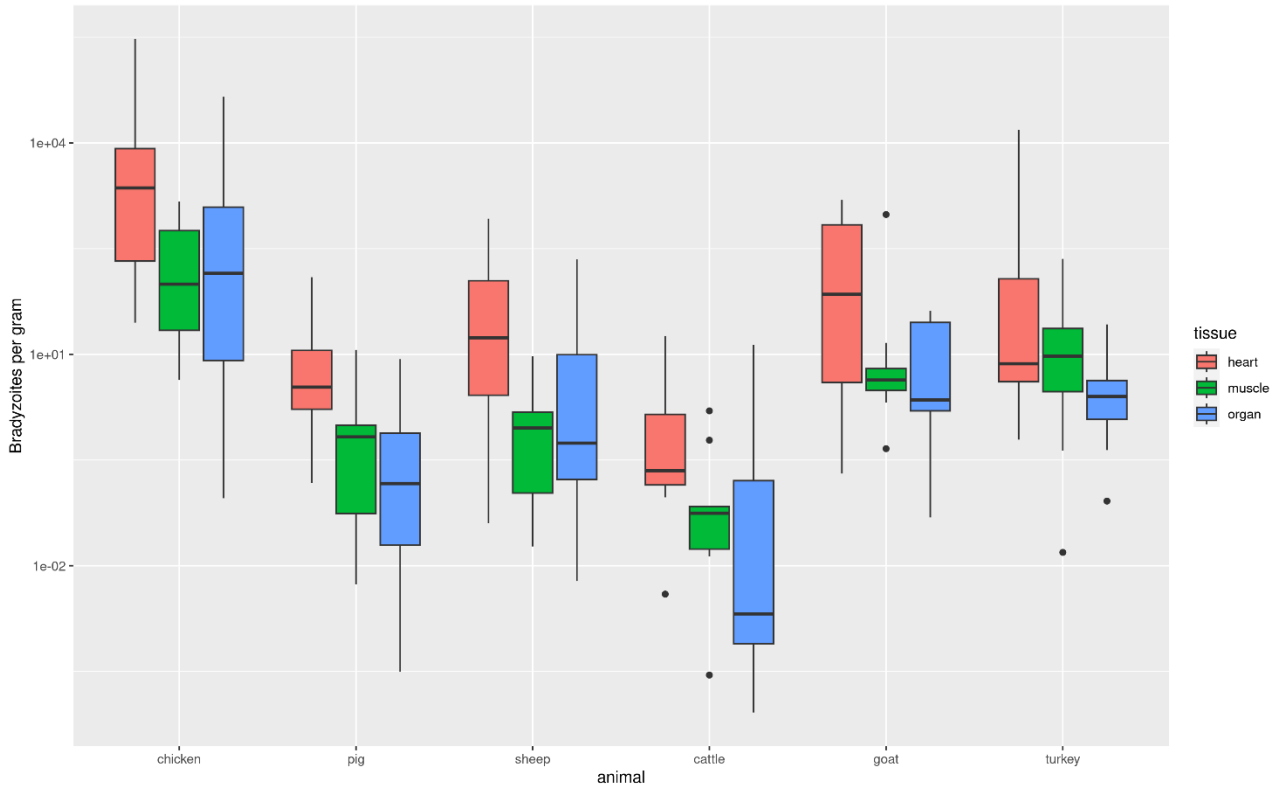

Figure 2. The anatomical distribution of bradyzoites in tissues of animals, model output for natural infection.

3. Consumer Survey

An online questionnaire with 34 multiple-choice questions was distributed by a market research agency among consumer panels in DE, DK, FR, NL, NO, PT, ES, CZ. The targeted number of respondents was 2000, except DK and NO with 3000 respondents each. The sample was drawn representative of region and education level. Respondents answered questions on the frequency of consumption and portion sizes for a range of meat products and raw vegetables, on specific risk behavior such as tasting raw meat and drinking unpasteurized milk, on heating preferences, storage conditions and washing of vegetables, and on buying organic meat and ready to eat vegetables.

The data were weighted by age and gender. Bayesian statistics were employed to derive probability distributions for each question. Consumption frequencies and preferences of preparation and storage were used in the model to define consumer behaviour.

4. Processing Parameters

Processing information for relevant generic and country-specific meat products was collected from handbooks, recipes, product label information, etc. by consortium members from all countries. Most of these parameters were characterized by a minimum and maximum value, enabling the use of probability distributions to describe variability over individual products. Included were: salting times, temperatures and concentration, heating times and temperatures, animals and tissues included in the product, edible fractions, and fat percentage. The development of this database was a consortium wide effort, where each participating country supplied information on their own speciality products. This work also included collaboration with the industry. The processing parameters are an input to the model, determining how the products that are to be consumed will be treated prior to consumption.

5. Inactivation Models

Three types of inactivation are implemented in the model, due to 1) heating 2) salting and 3) freezing. For heating we developed a model based on heat transfer in idealized geometrical shapes (slab, cylinder, sphere), that are meant to mimic meat products (e.g. steak, sausage, meatball). Heat transfer throughout the product was calculated using the well established heat equation. Inactivation of bradyzoites was modelled using published data on mouse survival after feeding of heat treated portions of infected meat. For the salting model, we improved upon an earlier model, and calculated reduction factors based on duration of salting, temperature, NaCl percentage and salting method (such as brining, injection, curing, etc.). The result of the salting model is shown in Figure 3. For freezing, complete inactivation was assumed.

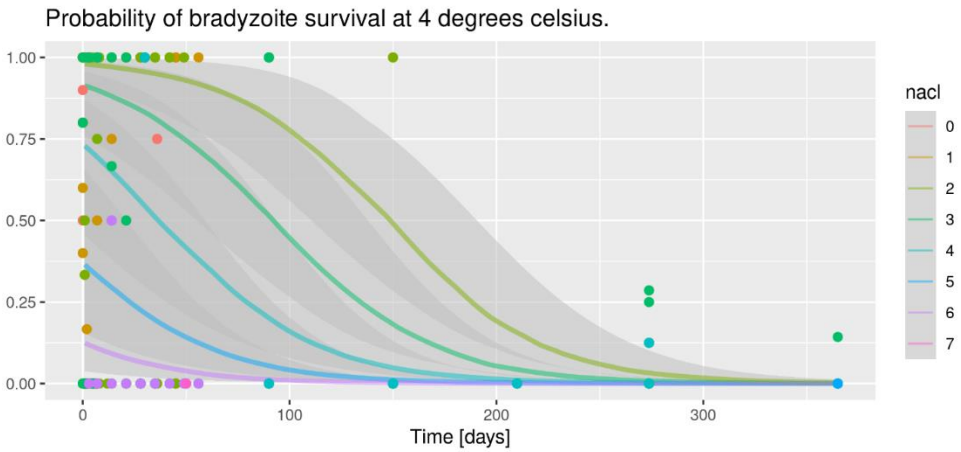

Figure 3.Probability of bradyzoite survival, at several salting levels (%nacl). Curves are model output with uncertainty intervals. Overlaid is the probability of mouse infection from the data. Note that there is not a one-to-one correspondence between mouse survival and bradyzoite inactivation, since mice can also remain uninfected at low dose.

6. Dose Response

The dose response is based on literature data on feeding experiments for several animal species (such as mice, pigs and cats). After fitting the Beta-binomial dose-response model, it is observed that the dose-response curves were not systematically different for any animal species, and hence we decided to pool the data. We obtain a dose-response relation an ID50 (dose at which 50% of the hosts get infected) of about 60 bradyzoites.

7. Model Output

T. gondii infection in humans is generally assumed to be for life. Hence, as an outcome measure we adopted the age of first infection.

Figure 4 shows an example of preliminary result of model outcome from one of the countries. In this outcome, 94 out of 100 persons were infected in their lifetime, which would be an overestimation of the true infection burden. However, the products that were identified by this model outcome have previously been identified as risk-products for the infection.

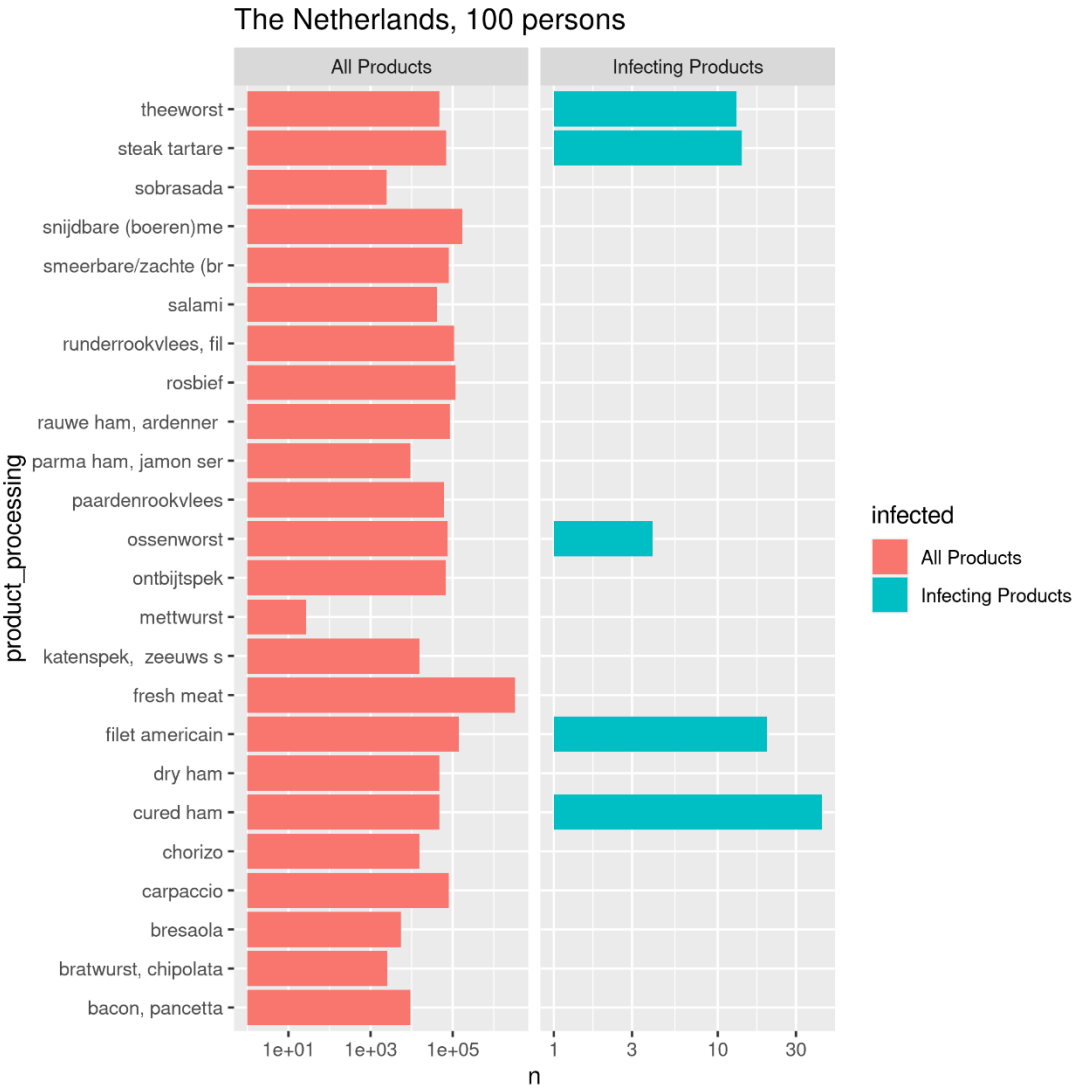

Figure 4. Meat products eaten by 100 persons in one year in the Netherlands (left panel) in log-scale, and products which the model indicated to have caused an infection (right panel).

DISCUSSION AND PERSPECTIVES

A quantitative microbiological risk assessment for *T. gondii* infection in nine European countries was successfully carried out. The data informing the model and the modelling approaches were improved substantially. In particular, the modelled age at first infection allows easy comparison with available seroprevalence by age data.

Identified high risk products are important for individual consumer decisions, and for public health decision making e.g. for making recommendations. On the population level, the importance of each product is influenced by frequency of consumption. Products with a large contribution on population level should be the targeted by intervention strategies, and the QMRA model can aid decision makers by providing risk-based estimates of the effect of possible interventions. The design of interventions will require a holistic and multidisciplinary approach. For example, high salt concentration reduces the risk of infection from unheated

meat products. However, high salt concentration is not in line with the efforts to reduce salt intake. Moreover, involvement of behavioral sciences and social sciences will be needed.

DISSEMINATION AND IMPACT

The work was performed successfully as an international collaboration of scientists from public health, animal health and food safety institutes, and the complementary expertise in the multidisciplinary TOXOSOURCES consortium proved useful in the process. During the whole process, regular meetings were organized to discuss the work, and the experiences were shared especially with TOXOSOURCES-WP3. The work included integrative aspects in terms of collaboration and harmonizing the process, and cross-sectoral aspects in terms of collaboration within the consortium, in particular with WP3. Capacity-building and training aspects were also included, and both experienced and early-career scientists participated in the work.

This work is an excellent example of efficient sharing of results within the TOXOSOURCES consortium to enable synergies and timely use of the results. Input data on fresh produce were made available by WP3, Prevalence data in animals by WP2-T2, consumption and preparation data by WP2-T3, meat processing data by WP2-T4, and data on prevalence and risk factors of *T. gondii* infection in humans by WP2-T5. All the model compartments (Figure 1) were defined using probability distribution functions, and can be reused allowing other research groups to update and build on newly acquired knowledge. This can be important for initiatives and projects on other pathogens sharing similar transmission routes.

Dissemination of the outcomes is ongoing in collaboration with TOXOSOURCES WP1 and following the FAIR principles. The work and its results are being prepared for peer-reviewed scientific publication and the manuscript will be submitted to an Open Access journal. A simplified food-chain model focusing on the meat processing part of the full QMRA model was presented at ApicoWplexa 2022 (5-7 October, Bern, Switzerland) by Marieke Opsteegh: 'Meat processing as part of a quantitative microbial risk assessment for *Toxoplasma gondii* infection in Europe'.

REFERENCES

Deng, H., Exel, K.E., Swart, A., Bonacic Marinovic, A.A., Dam-Deisz, C., van der Giessen, J.W.B., Opsteegh, M., 2021. Digging into Toxoplasma gondii infections via soil: A quantitative microbial risk assessment approach. Sci Total Environ 755, 143232.

Deng, H., Swart, A., Bonacic Marinovic, A.A., van der Giessen, J.W.B., Opsteegh, M., 2019. The effect of salting on Toxoplasma gondii viability evaluated and implemented in a quantitative risk assessment of meat-borne human infection. Int J Food Microbiol 314, 108380.

Opsteegh, M., Prickaerts, S., Frankena, K., Evers, E.G., 2011. A quantitative microbial risk assessment for meatborne Toxoplasma gondii infection in The Netherlands. International Journal of Food Microbiology 150, 103-114.