

ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD
UPDATE ON THE AGENCY'S WORK ON THE RELATIONSHIP BETWEEN
SEROPREVALENCE IN THE MAIN LIVESTOCK SPECIES AND THE
PRESENCE OF *TOXOPLASMA GONDII* IN MEAT

Introduction

1. Toxoplasmosis is a zoonotic disease with a worldwide distribution caused by the protozoan parasite *Toxoplasma gondii* (*T. gondii*). Humans can become infected via three main routes: (i) congenital following infection during pregnancy, (ii) ingestion of oocysts present in soil, water, vegetables or anything that has been in contact with cat faeces or, (iii) consumption of raw or undercooked meat containing *T. gondii* bradyzoites in tissue cysts ('infective cysts') [1, 2]. Although most infections in humans are asymptomatic, immunocompromised people are at higher risk of becoming seriously ill, whilst infection during pregnancy could result in ocular and neurological lifelong complications for the newborn.[1]
2. Ingestion of raw or undercooked meat has been suggested to be a major source of *T. gondii* infection in Europe and North America [3, 4], and in recent years, toxoplasmosis has been ranked as posing the highest disease burden among foodborne pathogens in the Netherlands and in the USA [5, 6], generating global concern and leading agencies such as the European Food Safety Authority to conduct consultations and issue scientific opinions on the risk that the parasite poses to human health.
3. In September 2012, the ACMSF published a 'Risk profile in relation to *Toxoplasma* in the food chain' which reviewed the evidence on toxoplasmosis in humans and animals and food particularly in the UK.
4. The recommendations made by the committee in the 2012 report relate to the data gaps identified in the risk profile which would inform risk assessment and appropriate risk management measures.
5. In 2013 EFSA launched a project (GP/EFSA/BIOHAZ/2013/01) to collect information on the presence and infectivity of *T.gondii* cysts in meat and other edible tissues (in the main meat-producing animals), and its relationship with *T.gondii* seroprevalence in animals.
6. The FSA joined the consortium of 12 organisations investigating the topic in the EU countries. On the UK-specific tasks FSA worked in close collaboration with RVC and the Moredun Institute.

7. The information on the initial phase of the project was included in ACM/1151 in June 2014 when the Committee were provided with a response to some of the recommendations in their report.

Evidence reflecting ACMSF recommendations

8. Lack of information regarding (i) seropositivity in meat-producing animals and presence of cysts and (ii) level of infection in livestock reared in the UK and potential risk factors associated with *T. gondii* infection of pigs were highlighted, among others, as important data gaps for the assessment of the risk of meat to human infection by the committee in 2012 [7].

Relationship between serology and presence of viable cysts in meat

9. Presence of antibodies in meat-producing animals is often used as an indirect indicator for the potential presence of infective cysts in meat.
10. Available evidence regarding relationship between seropositivity in meat-producing animals and the presence of cysts has been collected and synthesised in an extensive systematic literature review [8]. The report and supporting documentation is available on the EFSA web www.efsa.europa.eu .
 - Overall, a fair to moderate concordance was found in pigs, small ruminants and chickens between detection of antibodies to *T. gondii* and direct detection (bioassay or molecular methods) of the parasite. In contrast, data available on cattle and horses suggest a lack of concordance between serology and presence of *T. gondii* in these species, with a low recovery rate of the parasite in seropositive animals and similar rates of direct detection of the parasite in both seronegative and seropositive animals.
11. As part of the EFSA project, further evidence was generated from field studies in several countries for those species for which information was scarce or inconclusive [9].
 - The correlation between indirect detection (serology) and direct detection (molecular methods and bioassay) of *T. gondii* was assessed in cattle in a slaughterhouse-based study conducted in four countries: The Netherlands (NL), United Kingdom (UK), Romania (RO), and Italy (IT).
 - The study findings suggested a lack of correlation between seropositivity in cattle and presence of cysts in meat and, building on previous evidence. Therefore, serology has a limited value as an indirect indicator for the potential presence of infective cysts in beef and should not be used as a proxy for presence of cysts in meat. Diagnostic tests detecting *T. gondii* DNA or viable cysts should be used instead.

- In pigs, sheep and poultry, serological results can be used as an indicator for the potential presence of infective cysts in meat.

Level of infection in cattle in the UK

12. An abattoir survey conducted independently from the EFSA project, was undertaken between October 2015 and January 2016 to assess the level of infection by *T. gondii* in cattle raised and slaughtered in the UK for human consumption.
13. Given the lack of correlation between serology and presence of cysts in meat, molecular methods (magnetic capture qPCR (MC-PCR) and real time PCR) were used to assess the level of infection.
14. Overall, 305 animals were tested and their movement history, age, sex and breed was obtained using the British Cattle Movement System. The 305 animals sampled (41.6% females and 58.4% males) covered 614 different farms and 40 livestock markets across the country.
15. The true prevalence was estimated to be 1.79% (95% C.I. 1.66% - 1.95%).
16. Although extrapolation should be made with caution given the non-probabilistic selection of farms, the results from this study suggested a low level of infection in cattle raised and slaughtered in the UK with no clear geographic pattern of positive animals.

Level of infection and risk factors in pigs

17. A cross sectional study was conducted between January and July 2015 to assess the level of exposure to *T. gondii* in pigs raised in England and to identify factors associated with a higher risk of exposure.
18. In pigs, a fairly good correlation has been reported between seropositivity and presence of cysts (see above), therefore presence of antibodies was used to assess the level of infection.
19. A total of 2071 slaughter pigs originating from 131 farms were sampled and 75 (3.6%) were found to be seropositive by modified agglutination test (MAT). Using an empirical Bayes model, the estimated farm-level prevalence was 11.5% (95% C.I. 8.4%-16.0%). Data on potential risk factors was obtained for 73 farms that returned a completed questionnaire.
20. The risk of *T. gondii* infection increases 2.6 fold (p-value=0.04) in those farms where cats could gain access to the pigs' feed; 3 fold on those farms that allow outdoor access of pigs (p-value =0.04) and 3.9 fold on those farms keeping ≤ 200 pigs (p-value =0.02). Most batches were likely to contain 100% of uninfected pigs and the evidence suggests that infected pigs tend to concentrate in individual batches. Infection is therefore largely driven by farm-level factors and can be mitigated by farm-level measures that prevent cats gaining access to pigs and their feed.

Additional evidence

Predilection sites (i.e. the most likely tissues where cysts occur, as manifestations of a disease, condition, or presence of the pathogen)

21. Given the limited number of studies reporting predilection sites in cattle, an experimental study was conducted, to determine the dissemination of *T. gondii* cysts to different organs in cattle following infection by oral inoculation with *T. gondii* oocysts. Six Holstein Friesian calves were infected and followed up for 6 weeks. Serum samples were collected weekly. Once euthanized at 6 weeks, 100g tissue pools were prepared and tested using mouse bioassay and molecular methods (MC-PCR).
22. After slaughter, viable *T. gondii* and DNA were detected in various tissues and meat cuts. Many tissues tested positive to bioassay and MC-PCR (including *M. semitendinosus* and tongue). Although no clear predilection sites were identified, this study builds on evidence that cattle can become infected and develop infective cysts.
23. In calves no clear predilection sites were identified after oral inoculation with *T. gondii*.
24. There was little variation in parasite load between the different edible tissues (i.e. skeletal muscles) in experimentally infected pigs and sheep [9].

Vaccination

25. Vaccination with the S48 strain tachyzoites was shown to reduce tissue cyst formation in vaccinated pigs [7] and sheep.

Risk Assessment

26. Estimates of the relative importance of different meat products as a source of human exposure to *T. gondii* have been made available for Australia, The Netherlands and the US [10-12] and have informed positions arguing for a prominent role of the foodborne route of infection. A critical review of available risk assessments has revealed that they are based on a number of critical assumptions for which the data available are very limited.
27. Epidemiological investigations and experimental studies described above have filled in some of the knowledge gaps previously identified by the committee as necessary in order to carry out a quantitative risk assessment in the UK.
28. Data on the level of exposure in pigs and cattle are now available and can be used to populate the first stages of probabilistic risk assessments.

29. Nonetheless, key knowledge gaps still remain, continuing to undermine effective quantitative risk assessments and limiting the practical value for policy makers.
30. The number and distribution of viable cysts in meat and tissues of infected animals and the number of parasites (bradyzoites) per cyst remain important knowledge gaps.
31. Beyond assessment of the exposure, the lack of dose-response information for humans still represents an important limitation in making a final assessment of risk to human health.
32. Although not part of the original project plan, a stochastic model has been conceptualised highlighting those parameters and relationships of the variables involved that are still characterized by limited or missing information.
33. The stochastic model developed captures the biological dynamics of *T. gondii* and demonstrates that with the current level of data available quantitative estimations are characterised by high levels of uncertainty and should be interpreted with caution.
34. However, the 'ready-to-use' model developed can be used as key data become available. The model has been described in detail in a scientific paper which is currently being reviewed.

Suggestions for further activities

35. Promotion of primary production practices that minimize risk of on-farm exposure through industry (e.g. AHDB-pork). Prevention of *T. gondii* infection in pigs should be based on avoiding food contamination with cat faeces. The current guidance/advice to farmers should therefore emphasise the importance of ensuring that cats do not have access to pigs' feed.
36. It would be useful to implement some monitoring of the level of infection in pigs raised in the UK and entering the food chain. Given the low level of infection and the associated risk factors, a risk based strategy, targeting farms with outdoor access and/or open feed storage, would seem prudent.
37. To build and update evidence on the likely role of the different infection routes it will be important to engage with Public Health England surveillance activities for toxoplasmosis to gather the necessary data from confirmed cases. This could allow the investigation of factors associated with a higher risk of exposure including meat consumption and cooking habits and to assess the relative role of the foodborne routes of infection.

The Committee is asked:

- To note the findings of the research that was referred to in ACM/1151 (June 2014)
- To comment on the proposed suggestions for further activities
- To advise whether there are any other areas that need to be addressed.

References

1. Andreoletti, O., et al., *Surveillance and monitoring of Toxoplasma in humans, food and animals. Scientific opinion of the Panel on Biological Hazards*. The EFSA journal, 2007. **583**: p. 1-64.
2. Tenter, A.M., A.R. Heckeroth, and L.M. Weiss, *Toxoplasma gondii: from animals to humans*. International Journal of Parasitology, 2000. **30**: p. 1217-1258.
3. Cook, A.J.C., et al., *Sources of Toxoplasma infection in pregnant women: European multicentre case control study*. BMJ, 2000.
4. Flatt, A. and N. Shetty, *Seroprevalence and risk factors for toxoplasmosis among antenatal women in London: a re-examination of risk in an ethnically diverse population*. European Journal of Public Health, 2012. **23**: p. 648-652.
5. Batz, M.B., S. Hoffmann, and J.G. Morris, *Ranking the risks: the 10 pathogen-food combination with the greatest burden on Public Health*, Emerging Pathogens Institute, Editor. 2011, University of Florida: USA.
6. Havelaar, A.H., et al., *Disease burden of foodborne pathogens in the Netherlands*. International Journal for Food Microbiology, 2012. **156**: p. 231-238.
7. Burrells, A., et al., *Vaccination of pigs with the S48 strain of Toxoplasma gondii - safer meat for human consumption*. Veterinary Research, 2015. **46:47**.
8. EFSA. Relationship between seroprevalence in the main livestock species and presence of Toxoplasma gondii in meat (GP/EFSA/BIOHAZ/2013/01) An extensive literature review. Final report 2016. Accessed on 10 June 2016. <http://www.efsa.europa.eu/en/supporting/pub/996e>
9. EFSA, Experimental studies on Toxoplasma gondii in the main livestock species (GP/EFSA/BIOHAZ/2013/01) Final report 2016. Accessed on 10 June 2016. <http://www.efsa.europa.eu/en/supporting/pub/995e>
10. Guo, M., et al., *Qualitative Assessment for Toxoplasma gondii Exposure Risk Associated with Meat Products in the United States*. Journal of Food Protection, 2015. **78**: p. 2207-2219.
11. Mie, T., et al., *A qualitative assessment of Toxoplasma gondii risk in ready-to-eat smallgoods processing* Journal of Food Protection, 2008. **71**: p. 1442-1452.
12. Opsteegh, M., et al., *A quantitative microbial risk assessment for meatborne Toxoplasma gondii infection in The Netherlands*. International Journal for Food Microbiology, 2011. **150**: p. 103-114.

**Secretariat
June 2016**

Published and planned publications generated by the project

Opsteegh, M., Maas, M., Schares, G., van der Giessen, J., on behalf of the consortium. 2016, Relationship between seroprevalence in the main livestock species and presence of *Toxoplasma gondii* in meat (GP/EFSA/BIOHAZ/2013/01). An extensive literature review. Final Report. EFSA supporting publication.

Opsteegh, M., Schares, G., Blaga, R., van der Giessen, J., on behalf of the consortium. 2016, Experimental studies on *Toxoplasma gondii* in the main livestock species (GP/EFSA/BIOHAZ/2013/01). Final Report. EFSA supporting publication.

Limon, G., Burrells, A., Dadios, N., Hosein, S., Vince, L., Crotta, M., Blake, D., Katzer, F., Guitian, J., 2016, *Toxoplasma gondii*: Relationship between seroprevalence and presence in meat, level exposure in UK pigs and cattle and hypothetical human exposure assessment. FSA final report (FS517004).

Limon, G., Beauvais, W., Dadios, N., Villena, I., Cockle, C., Blaga, R., Guitian, J., Cross sectional study of *Toxoplasma gondii* infection in pig farms in England, *submitted to Zoonoses and Public Health*.

Hosein, S., Limon, G., Dadios, N., Guitian, J., Blake, D. P., *Toxoplasma gondii* detection in UK cattle: A slaughterhouse survey, *in preparation*

Crotta, M., Limon, G., Blake, D., Guitian, J., A hypothetical model for the assessment of human exposure to *Toxoplasma gondii* through consumption of meat products, *in preparation*