Ministry for Primary Industries
Manatū Ahu Matua

Risk Profile Update: Toxoplasma Gondii in Red Meat and Meat Products

MPI Technical Paper No: 2015/33

ISBN No: 978-1-77665-066-8 (online)
ISSN No: 2253-3923 (online)

July 2015

New Zealand Government

Growing and Protecting New Zealand

RISK PROFILE UPDATE:
TOXOPLASMA GONDII
IN RED MEAT AND MEAT PRODUCTS

Client report FW15009

By

Peter Cressey
Dr Rob Lake

July 2015

Institute of Environmental Science & Research Limited
Christchurch Science Centre
Location address: 27 Creyke Road, Ilam, Christchurch
Postal address: P O Box 29 181, Christchurch, New Zealand
Website: www.esr.cri.nz

A CROWN RESEARCH INSTITUTE

RISK PROFILE:
TOXOPLASMA GONDII
IN RED MEAT AND MEAT PRODUCTS

Prepared for the Ministry for Primary Industries
under project MRP/14/01 - Microbiological Risk Profiles,
as part of an overall contract for scientific services

Client report no. FW15009

Dr Stephen On
Chief Scientist, Environmental Science

Dr Rob Lake
Project Leader

Dr Lucia Rivas
Peer Reviewer

DISCLAIMER

This report or document ("the Report") is given by the Institute of Environmental Science and Research Limited ("ESR") solely for the benefit of the Ministry for Primary Industries ("MPI"), Public Health Services Providers and other Third Party Beneficiaries as defined in the Contract between ESR and MPI, and is strictly subject to the conditions laid out in that Contract.

Neither ESR nor any of its employees makes any warranty, express or implied, or assumes any legal liability or responsibility for use of the Report or its contents by any other person or organisation.

GOVERNMENT AGENCY MERGERS AFFECTING THIS DOCUMENT

On 1 July 2010, the New Zealand Food Safety Authority (NZFSA) and the Ministry of Agriculture and Forestry (MAF) were amalgamated. On 30 April 2012, MAF was renamed as the Ministry for Primary Industries (MPI).

This Risk Profile uses the names NZFSA and MAF for documents produced during the existence of these organisations.

ACKNOWLEDGMENTS

The authors wish to acknowledge the Ministry of Health as owner of the copyright and funders of the 1997 National Nutrition Survey, and the 2002 National Children's Nutrition Survey and the 2009 Adult Nutrition Survey, and to thank them for access to food consumption information (24-hour dietary recall and qualitative food frequency questionnaire) from these surveys.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

GLOSSARY AND ABBREVIATIONS

ANS	The 2009 Adult Nutrition Survey
a_w	Measure of water activity (max = 1.000 = pure distilled water)
ACMSF	Advisory Committee on Microbiological Safety of Foods
CFU	Colony forming unit
CNS	The 2002 National Children's Nutrition Survey
EU	European Union
FAO	Food and Agriculture Organisation of the United Nations
FSANZ	Food Standards Australia New Zealand
MPI	Ministry for Primary Industries
MPN	Most Probable Number
Neonate	A newborn baby during the first 28 days after birth
NNS	The 1997 National Nutrition Survey
NZFSA	New Zealand Food Safety Authority (now MPI)
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
pH	Measure of acidity (min = 0 = most acidic; max = 14)
Perinatal	The period from 20 weeks or more gestation to 7 days after birth
RMP	Risk Management Programme (under the *Animal Products Act* 1999)
RTE	Ready-to-eat
USA	United States of America
USFDA	United States Food and Drug Administration
USDA	United States Department of Agriculture
VBNC	Viable but non-culturable
WHO	World Health Organisation

SUMMARY

This Risk Profile considers *Toxoplasma gondii* in red meat and meat products. This is an update of a Risk Profile "*Toxoplasma gondii* in red meat and meat products" published in 2008 (Lake *et al.*, 2008).

The purpose of the current Risk Profile is to critically review new information to answer the following risk management questions:

- What is the public health risk from *T. gondii* in red meat and meat products consumed in New Zealand?
- Has the risk of toxoplasmosis, from the consumption of red meat and meat products, consumed in New Zealand changed since the 2008 Risk Profile?

T. gondii is a protozoan parasite. Cysts or eggs are shed with faeces of the reservoir definitive host (in this case members of the cat family), in which the organism is able to reproduce sexually in large numbers. Excreted organisms are able to infect intermediate hosts such as warm-blooded animals (including humans) and birds, in which they form tissue cysts. The intermediate hosts are often also major food sources for humans, including livestock providing red meat.

Red meat production remains a major industry in New Zealand. While numbers of animals of the major red meat species (sheep, beef cattle, deer and pigs) farmed in New Zealand have decreased in the last decade, this is unlikely to have implications for the Risk Profile.

Measures such as heating, freezing and irradiation are effective in inactivating *T. gondii*. Variables in the production of processed meats (e.g. pH, salt and preservatives) appear to have an impact on the survival on *T. gondii*, but further definition of the exact conditions required for inactivation is desirable.

No information is available on the prevalence of *T. gondii* in red meat and red meat products in New Zealand. The limited information on seroprevalence in New Zealand meat-producing animals (sheep, deer and goats) suggests that they are regularly exposed to *T. gondii*. This is consistent with the fact that most meat-producing animals in New Zealand have access to the outside environment and probable contact with oocysts. No information is available on the seroprevalence of *T. gondii* in cattle or pigs in New Zealand.

Red meat and red meat products are commonly consumed foods in New Zealand. Decreases in the frequency of consumption, as assessed through national Nutrition Surveys, has been paralleled by increases in serving sizes. There is no difference in consumption patterns of beef and sheepmeat by pregnant women, but some indications that they consume pigmeat less frequently than the general population.

The burden of toxoplasmosis in New Zealand can only be assessed through occasional seroprevalence studies and hospital discharges associated with congenital or acquired toxoplasmosis. The two seroprevalence studies reported since the last version of this Risk Profile give very similar estimates of seroprevalence in the adult population. These estimates are within the range of seroprevalence estimates found in earlier New Zealand studies.

A quantitative risk assessment for foodborne toxoplasmosis in New Zealand is not available. Estimated attribution of toxoplasmosis to foodborne transmission in New Zealand (approximately 30%) is similar to estimates from other developed countries.

The information on *T. gondii* in red meat and meat products in New Zealand since the previous Risk Profile is limited, and essentially the conclusions in the previous document still apply.

Expert opinion in New Zealand attributes 30% of transmission to foodborne routes. Apart from meat, fruits and vegetables, unpasteurised milk and possibly shellfish, are potential transmission vehicles. Given that infective cysts are readily destroyed by cooking and freezing, exposure from red meat appears unlikely to be the major foodborne transmission pathway. No information was found on the prevalence of raw red meat consumption in New Zealand.

There remains considerable methodological barriers to evaluating the risk of *T. gondii*, such as determining the infectivity status of contamination in meat and determining the prevalence and burden of disease. We consider that further work is justified on the risk from *T. gondii* in red meat, but initially, the need is to characterise the burden of toxoplasmosis in New Zealand.

1 INTRODUCTION

Risk Profiles provide scientific information relevant to a food/hazard combination for risk managers and describe potential risk management options.[1] This document updates a previous Risk Profile considering *Toxoplasma gondii* in red meat and meat products which covered information up to 2006 (Lake *et al.*, 2008).

The purpose of this update is to critically review new information to answer the following risk management questions:

- What is the public health risk from *T. gondii* in red meat and meat products consumed in New Zealand?
- Has the risk of toxoplasmosis, from the consumption of red meat and meat products, consumed in New Zealand changed since the previous Risk Profile?

The current Risk Profile therefore reviews data relevant to the scope which has been published from 2006 to date.

[1] http://foodsafety.govt.nz/elibrary/industry/RMF_full_document_-_11604_NZFSA_Risk_Management_Framework_3.1.pdf accessed 27 August 2014

2 HAZARD AND FOOD

2.1 The Pathogen: *Toxoplasma gondii*

KEY FINDINGS

T. gondii is a protozoan parasite. Cysts or eggs are shed with faeces of the reservoir definitive host (in this case members of the cat family), in which the organism is able to reproduce sexually in large numbers. Excreted organisms are able to infect intermediate hosts such as warm-blooded animals (including humans) and birds, in which they form tissue cysts. The intermediate hosts are often also major food sources for humans, including livestock providing red meat.

Appendix 1 contains additional information on T gondii.

2.1.1 Disease and pathogenicity

T. gondii is an obligate intracellular protozoan parasite. The organism is able to reproduce sexually to high numbers in the definitive host (members of the cat family). Cysts are then shed with faeces of the reservoir definitive host. Excreted organisms are able to infect intermediate hosts such as warm-blooded animals (including humans) and birds, where the organism is able to replicate asexually and form tissue cysts. The intermediate hosts are often also major food sources for humans, including livestock providing red meat (Goldsmid *et al.*, 2003).

T. gondii is considered to have three stages of infection; the oocyst environmental stage, the tachyzoite stage of rapid division, and the bradyzoite stage of slow division within tissue cysts (see Appendix 1, section 8.1 for more detail on the life cycle of *T. gondii*) (Robert-Gangneux and Darde, 2012). When an oocyst or tissue cyst is ingested by a human or other warm-blooded animal, the resilient cyst wall is dissolved by proteolytic enzymes in the stomach and small intestine, freeing sporozoites from within the oocyst or bradyzoites from within the tissue cyst. The parasites first invade cells in and surrounding the intestinal epithelium, and inside these cells, the parasites differentiate into tachyzoites, the motile and quickly multiplying cellular stage of *T. gondii*. Tissue cysts in tissues such as brain and muscle tissue, form approximately 7–10 days after initial infection.

Once inside the host cell, the tachyzoites replicate until the host cell dies and ruptures, releasing and spreading the tachyzoites via the bloodstream to all organs and tissues of the body, including the brain.

Following the initial period of infection characterised by tachyzoite proliferation throughout the body, pressure from the host's immune system causes *T. gondii* tachyzoites to convert into bradyzoites, the semidormant, slowly-dividing cellular stage of the parasite. Inside the host cell, clusters of these bradyzoites are known as tissue cysts. Upon ingestion of these tissue cysts by an intermediate host through raw or undercooked meat, cysts are ruptured as they pass through the digestive tract, causing the release of bradyzoites. These will infect the intestinal epithelium of the new host and differentiate back into the rapidly dividing tachyzoite stage for dissemination throughout the body (Robert-Gangneux and Darde, 2012).

The multiple infective forms of the organism offer opportunities for elucidating the epidemiology of the disease. An analytical method has been developed that targets antibodies to a specific 11 kDa sporozoites protein (Hill *et al.*, 2011). This enables an assessment of whether an infection has occurred through ingestion of oocysts (sporozoites-producing), rather than tissue cysts (bradyzoites-producing). With respect to the topic of the current Risk Profile, bradyzoites stage infections are likely to be specific to consumption of meat from infected animals. Although sporozoites stage infections may be due to consumption of meat contaminated by oocysts from the environment, they are more likely to be due to a range of other food or environmental exposures.

2.2 The Food: Red Meat and Meat Products

KEY FINDINGS

Red meat production remains a major industry in New Zealand. While numbers of the major red meat species farmed in New Zealand have decreased in the last decade, this is unlikely to have implications for the Risk Profile.

2.2.1 Definitions

For the purpose of this Risk Profile 'red meat' is taken to include the skeletal muscular tissue and associated materials (fat and other tissues) from the main commercial meat species (i.e. cattle, sheep, pigs and deer). This Risk Profile also addresses veal, which is the meat of very young cattle (calves).

2.2.2 The food supply in New Zealand: Red meat and red meat products

2.2.2.1 The red meat industry

The Meat Industry Association of New Zealand (MIA) is a voluntary trade association representing New Zealand meat processors, marketers and exporters. MIA member companies operate approximately 60 processing plants throughout the country, representing companies supplying the majority of New Zealand sheep and beef meat exports. Sheep meat and beef exports make up 13% of New Zealand's exports by value (22% of New Zealand's primary sector revenue).[2] For the year ending June 2014, the meat industry earned export revenue of $5.8 billion.[3]

2.2.2.2 Production

New Zealand is a major producer and exporter of red meat. Between 2002 and 2014, sheep numbers in New Zealand decreased from approximately 40 million to just under 30 million, beef cattle numbers decreased from 4.5 million to 3.6 million, deer numbers decreased from 1.6 million to 950,000 and pig numbers decreased from 342,000 to 285,000 (Statistics New Zealand, 2014). These decreases are at least partially due to a move to dairy farming, with the number of dairy cattle increasing during the same period from 5.2 million to 6.7 million.

[2] http://www.mia.co.nz/about_us/ Accessed 16 March 2015
[3] http://www.stats.govt.nz/infoshare/ Accessed 16 March 2015

Livestock slaughter and export statistics for the year ending 30 September 2013 are shown in Table 1.

Table 1: Livestock numbers, production and export for New Zealand, year ending 30 September 2013

Livestock type	Total inspected slaughter at export plants and abattoirs (million head)	Meat production, bone in basis (000 tonnes)	Meat exports (000 tonnes)
Lamb	21.3	382.4	313.1
Sheep	4.3	105.6 (mutton)	85.0
Cattle (including calves)	4.3	628.3 (beef and veal)	366.5 (beef and veal)
Pigs	0.7	47.1	0.1
Deer	0.4	22.9	13.2

Source: Compendium of New Zealand Farm Facts (Beef + Lamb New Zealand, 2014).

2.2.2.3 New Zealand exports

New Zealand is a major exporter of beef and sheep meat, with approximately 882,000 tonnes of meat and offals exported in the 12 months to 30 June 2014.[4] Approximately 85% of New Zealand's beef and sheepmeat production is exported.[5]

New Zealand exports only a small amount of pork (approximately 63 tonnes in 2014), mainly to Pacific Island nations (69%) and Hong Kong (29%).[6]

In the year ending September 2013, 15,428 tonnes of venison were exported (Beef + Lamb New Zealand, 2014).

2.2.2.4 New Zealand imports

New Zealand imports relatively small amounts of beef and sheep meat, according to data from Statistics New Zealand.[7] In 2014, approximately 9,400 tonnes of beef and 2,800 tonnes of sheep meat were imported from Australia. These quantities are greater than for 2012 (3,500 and 1,900 tonnes, respectively).

New Zealand's pigmeat supply is almost evenly divided between domestically produced and imported product. In the 2012/13 year, New Zealand's pigmeat supply was 93,718 tonnes, of which 46,517 tonnes were imported (Beef + Lamb New Zealand, 2014). In the year ending September 2014, domestic production was reported to be 47,646 tonnes (New Zealand Pork, 2014). Pigmeat was primarily imported from Canada (23%), USA (19%), Denmark (18%), Finland (16%), Australia (11%) and Sweden (8%).

[4] http://www.stats.govt.nz/infoshare/SelectVariables.aspx?pxID=e18ecf4c-dbbb-4ba7-ab3b-49752d253566 Accessed 23 October 2014

[5] http://www.mia.co.nz/about_us/ Accessed 16 March 2015

[6] http://www.stats.govt.nz/infoshare/TradeVariables.aspx?DataType=TEX accessed 186 March 2015

[7] http://www.stats.govt.nz/infoshare/TradeVariables.aspx?DataType=TIM accessed 27 June 2013

2.3 Behaviour of *T. gondii* in Red Meat and Meat Products

KEY FINDINGS

Measures such as heating, freezing and irradiation are effective in inactivating *T. gondii* in meat. Variables in the production of processed meats (e.g. pH, salt and preservatives) appear to have an impact on the survival on *T. gondii*, but further definition of the exact conditions required for inactivation is desirable.

More information is supplied in Appendix1

T. gondii may occur in meat in an encysted form (bradyzoites) or due to environmental contamination of the meat surface with sporozoites-containing oocysts. While *T. gondii* cysts may remain infective in meat, the organism will not grow outside of a live host.

2.3.1 Contamination of red meat and meat products by *T. gondii*

Raw sausage products (Teewurst, Mettwurst and salami) were manufactured from the meat of pigs that had been intravenously infected with *T. gondii* (10^4 tachyzoites) at three months of age (Abdulmawjood *et al.*, 2014). The presence of *T. gondii* DNA was confirmed in 30 out of 210 (14.3%) sausage samples by PCR. Infectivity was confirmed by feeding positive raw sausage material to mice and analysing organs (brain, heart and spleen) for *T. gondii* DNA by PCR after approximately 45 days. *T. gondii* DNA was detected in 4 out of 288 (1.4%) of mice. While raw sausage samples fed to the mice represented different processing variables (organic, without addition of nitrate, and conventional, with addition of nitrate) and ripening stages (from date of manufacture, date of marketing and end of shelf life), the low number of positive results meant no conclusions could be drawn on the impact of processing on *T. gondii* persistence in red meat products.

2.3.2 Inactivation of *T. gondii* in red meat and meat products

Meat from experimentally *T. gondii* infected goats was used to manufacture vacuum-packed goat meat and dry fermented goat sausages (Neumayerová *et al.*, 2014). Infectivity of meat products was assessed by mouse bioassay. Non-salted, vacuum-packed meat remained infective after storage at 4ºC for 42 days (the duration of the experiment). Freezing non-salted, vacuum-packed goat meat at -20ºC resulted in a loss of infectivity after 4 hours. Salted vacuum-packed goat meat stored at 4ºC remained infective after 7 days, but not after 14 days. The salt content of the meat was approximately 1.9%. No infectivity was observed in any sample of dry fermented goat sausage. The study authors suggested that this product's high salt content and low water activity is not amenable for *T. gondii* bradyzoite survival.

Pigs (n = 6) that were seropositive for *T. gondii* (IFAT titre ≥80) were slaughtered and used in the production of dry-cured hams (Bayarri *et al.*, 2010). Portions of ham were tested for *T. gondii* viability before curing (day zero), after 7 months, and at the completion of curing (14 months) by mouse bioassay (5 mice per test). All hams were negative before curing, all were positive at 7 months and all were negative at 14 months. The authors of the study suggested that the lack of viability before curing may have been due to the fact that only the surface of the hams was sampled for analysis at that time point, while the later time points sampled locations throughout the hams.

Mouse and cat bioassays were used to assess the effectiveness of a range of processing measures in inactivating *T. gondii* cysts in experimentally infected sheep meat (El-Nawawi *et al.*, 2008). No infectivity remained in the meat after heating at 60 or 100ºC for 10 minutes, but infectivity was still present after heating at either temperature for 5 minutes. Freezing at -10ºC for 3 days or -20ºC for 2 days was effective in inactivating tissue cysts. Viable cysts were still detected after freezing at -10ºC for 1 or 2 days or at -20ºC for 1 day. Irradiation with 75 or 100 kRad, but not 25 or 50 kRad, resulted in complete inactivation of cysts. However, cooking in a microwave (5 minutes on medium power) or chilling at 5ºC for 5 days did not completely inactivate cysts. It is uncertain whether lower periods of microwave cooking or chilling would inactivate *T. gondii* cysts, although earlier studies that involved cooking in a microwave for up to 15 minutes at maximum power and then at medium power until an internal temperature of 65ºC was achieved did not result in complete inactivation of *T. gondii* (Lundén and Uggla, 1992).

Freezing of meat has been reported to be an effective measure for inactivation of *T. gondii*. However, analysis of frozen buffalo meat illegally imported into Turkey found *T. gondii* tissue cysts in 3 of 20 (15%) samples by percoll density separation and light microscopy (Gencay *et al.*, 2013). *T. gondii* tissue cysts were not detected after refreezing of meat at -18ºC for two days. It should be noted that no assessment of the viability of encysted *T. gondii* was carried out.

Muscle and brain tissue from experimentally infected mice was placed in cell culture medium and treated with different regimes of pH, salt and nitrite for up to 30 days at 4ºC (Pott *et al.*, 2013). Infectivity was assessed by mouse bioassay. Tissue remained infective for at least 24 days at media with a pH range of 5-7. Cysts were more susceptible to salt, where infectivity remained after storage in a medium containing 2.0% salt for 8 days but higher salt concentrations (2.5 or 3.0%) resulted in loss of infectivity within 1 day. Salt in combination with 0.5% sodium nitrite was even more effective and no infectivity was detected after 4 days for cysts in a medium containing 2% salt and 0.5% sodium nitrite.

3 EXPOSURE ASSESSMENT

KEY FINDINGS

No information is available on the prevalence of *T. gondii* in red meat and red meat products in New Zealand. The limited information on seroprevalence in New Zealand meat-producing animals suggests that they are regularly exposed to *T. gondii*. This is consistent with the fact that most meat-producing animals in New Zealand have access to the outside environment and probable contact with oocysts, due to the high prevalence of cats (the definitive host) in New Zealand.

Red meat and red meat products are commonly consumed foods in New Zealand. Decreases in the frequency of consumption, as assessed through national Nutrition Surveys, has been paralleled by increases in serving sizes. There is no difference in consumption patterns of beef and sheepmeat by pregnant women, but some indications that they consume pigmeat less frequently than the general population.

3.1 New Zealand Prevalence Studies

As noted in the previous version of this Risk Profile, there are no data on the prevalence of *T. gondii* in red meat in New Zealand. Most of the information regarding the prevalence of *T. gondii* is derived from studies that measure antibodies against the organism present in the blood of humans and animals (seroprevalence).

The previous Risk Profile reported seroprevalences in deer (52.2%) and goats (37% in adults) but information for other species was only indirect. Since then a seroprevalence study has been carried out on blood samples (n = 2254) from unvaccinated ewe flocks, collected during the years 2006 to 2009 (Dempster *et al.*, 2011). *T. gondii* antibodies were detected using a latex agglutination (LAT) kit. Using a titre dilution cut-off[8] of 1:16, 1917/2254 (85.0%, 95th percentile confidence interval 83.5-86.5%) blood samples were positive. At a titre dilution cut-off of 1:64, 1384/2524 (61.4%, 95th percentile confidence interval 59.4-63.4%) were positive. The blood samples came from ewes in 198 flocks. All flocks were positive for *T. gondii* antibodies at a titre dilution cut-off of 1:16. It should be noted that these results were from a free screening service, with blood samples supplied by veterinarians wishing to assess the exposure of their clients' ewe flocks to *T. gondii*. This represents a potential selection bias for this sample set. It has been estimated that approximately 80% of breeding ewes in New Zealand are vaccinated against *T. gondii* infection (Sam Higgins, MSD Animal Health, Wellington, personal communication, April 2015). Vaccination is reported to inhibit cyst formation and it is likely that the overall seroprevalence of *T. gondii* in New Zealand breeding ewes is lower than determined in this survey.

3.2 Product Recalls

No recalls for the presence of *T. gondii* in meat or meat products have been reported in New Zealand in the period 2008 to the present.[9] However, this is not unexpected as no testing of food is conducted in New Zealand.

[8] The titre cutoff is the dilution at which a positive response is considered to represent a positive result
[9] http://www.foodsmart.govt.nz/food-safety/recalls/latest-recalls/ Accessed 9 March 2015

3.3 Requirements for Imported Food

There are currently no Imported Food Requirements (IFRs) related to the presence of *T. gondii* in any foods, including red meat and meat products (Ministry for Primary Industries, 2014).

3.4 Red Meat and Meat Product Consumption

Red meat and red meat products are commonly consumed foods in New Zealand. Table 2 summarises data on total beef, sheepmeat and pigmeat consumption by adult (15+ years) and child (5-14 years) New Zealanders. These figures are based on analysis of data (Cressey *et al.*, 2006; Cressey, 2013) from the 2009 Adult Nutrition Survey (2009ANS) (University of Otago and Ministry of Health, 2011) and the 2002 National Children's Nutrition Survey (Ministry of Health, 2003).

Table 2: Consumption of beef, sheepmeat and pigmeat by adult (15+ years) and child (5-14 years) New Zealanders

	Beef		Sheepmeat		Pigmeat	
	Adult	**Child**	**Adult**	**Child**	**Adult**	**Child**
Number of respondents	4721	3275	4721	3275	4721	3275
Percent consumers (%)	46.0	50.6	16.3	16.5	38.3	37.3
Serving per day (consumers)	1.3	1.3	1.1	1.3	1.3	1.4
Consumer mean (g/person/day)	106.8	85.4	79.7	77.2	71.8	53.3
Population mean (g/person/day)	49.1	43.2	13.0	12.8	27.5	19.9
Serving size, mean (g)	84.3	64.1	70.5	60.1	56.6	38.8
Serving size, median (g)	56.3	42.8	35.2	34.3	31.2	27.0
Serving size, 95th percentile (g)	242	184	228	178	185	117

The data in Table 1 confirm that there is little difference in the frequency of consumption (percent consumers) of the meat types between adult and child New Zealanders. As might be expected, adult serving sizes are, on average, greater than those consumed by children. Serving sizes for pigmeat are generally smaller than for the other meat types, reflecting the high proportion of processed meat products that are produced from pigmeat (e.g. ham and salami). Serving sizes for processed meat are generally smaller than for meat cuts.

Meat consumption patterns for adults can additionally be compared to those reported an earlier National Nutrition Survey (1997) to establish any trends in red meat consumption patterns (Russell *et al.*, 1999). Comparisons suggest a slight decrease in the frequency of beef consumption, while sheepmeat and pigmeat consumption has remained unchanged. However, for all meat types there is evidence that serving sizes have increased with time.

Of particular relevance to this risk profile, analysis of data from the 2009 Adult Nutrition Survey suggests no difference in the frequency and serving size of beef and sheepmeat consumption between pregnant women and the general adult population (Cressey, 2013). Pregnant women are significantly less likely to consume pigmeat than the general adult population, although there is no difference in average serving sizes.

3.5 Potential for Growth of *T. gondii* along the Red Meat and Meat Product Food Chain

T. gondii does not multiply outside its host environment. Consequently, growth will not occur in red meat and meat products (Mie *et al.*, 2008).

3.6 Data on *T. gondii* in Red Meat and Meat Products from Other Countries

KEY FINDINGS

Exposure of red meat-producing animals to *T. gondii* appears to be universal, on the basis of seroprevalence studies in a large number of countries. While fewer studies are available on the presence of infective *T. gondii* oocysts or tissue cysts in red meat and red meat products, it should probably be assumed that some proportion of these products will carry *T. gondii* infectivity, irrespective of the origin of the products.

Appendix 1 contains detailed data summarised in this section.

The vast majority of studies that provide information on the potential presence of *T. gondii* in red meat are seroprevalence studies on animals, either on farm or at slaughter. Results of studies published since the previous version of this Risk Profile are summarised in Table 5, Appendix 1. While seroprevalence varies considerably across studies, it broadly appears that seroprevalence is higher in sheep and goats, compared to cattle, pigs and deer.

Comparison between reported estimates of *T. gondii* seroprevalence in animals and in meat is complicated because of the multiplicity of analytical methods used and the variation in the application of methods between studies. For example, titre cutoffs (the dilution at which a positive response is considered to represent a positive result) for the same method can vary considerably. For example, the indirect fluorescence antibody test (IFAT) has been used applying cutoffs from 1:8 dilution (Górecki *et al.*, 2008) to 1:200 (Chandrawathani *et al.*, 2008; Fusco *et al.*, 2007).

The results of several studies reporting the prevalence of *T. gondii* in animal tissues or meat products are summarised in Table 5, Appendix 1. Although the results suggest that infective *T. gondii* is present in red meat and red meat products in many countries the prevalence of positive results by laboratory detection tests, such as PCR or ELISA is generally higher than infectivity determined by the mouse bioassay.

The mouse (or cat) bioassay remains the most definitive means of assessing the ability of animal tissues to cause *T. gondii* infection, either through the presence of oocysts or tissue cysts.

4 EVALUATION OF ADVERSE HEALTH EFFECTS

4.1 Disease Characteristics

KEY FINDINGS

There has been no change in the disease characteristics of toxoplasmosis since the previous version of this Risk Profile. *T. gondii* infections are asymptomatic in most healthy immunocompetent people and severe health effects are rare. High risk groups for toxoplasmosis are pregnant women and their foetuses, neonates and immunocompromised people. There is a developing body of evidence associating *T. gondii* infections with mental illness, specifically schizophrenia.

Appendix 2 contains detail on disease characteristics

Primary acquired infection is asymptomatic in more than 80% of cases of immunocompetent subjects in developed countries (Montoya and Liesenfeld, 2004). In the remaining cases patients may experience fever, cervical lymphadenopathy, sometimes associated with myalgia, asthenia or other nonspecific clinical signs. More rarely toxoplasmic chorioretinitis with visual impairment may occur (Robert-Gangneux and Darde, 2012). Severe life threatening toxoplasmosis occurs in immunocompromised patients, including those who are HIV positive or taking immunosuppressive therapies. Congenital infection may result from primary acquired maternal infection during gestation. Such vertical transmission is more likely as the pregnancy progresses, but foetal damage, when it occurs, is more severe in the early stages. Consequences can include severe abnormalities or abortion (Robert-Gangneux and Darde, 2012). Retinochorditis (inflammation of the retina) is a common feature, which frequently occurs in the child after birth.

There is increasing interest in possible associations between *T. gondii* infection and psychiatric disorders. A meta-analysis found a statistically significant association between schizophrenia and *T. gondii* infection (odds ratio 2.70, 95th percentile confidence interval 1.34-4.42, p = 0.005) (Arias *et al.*, 2012). The authors of this meta-analysis urged some caution, as the higher quality studies included in the analysis did not produce significant odds ratios. A second meta-analysis examining the association between the presence of *T. gondii* antibodies and schizophrenia estimated an almost identical odds ratio based on 23 studies (Torrey *et al.*, 2007). An updated meta-analysis by the same group considered an additional 15 studies and again estimated an odds ratio of approximately 2.7 (Torrey *et al.*, 2012). When compared to other risk factors for schizophrenia, *T. gondii* infection was considered to be an 'intermediate' risk factor.

4.2 Dose Response

KEY FINDINGS

No information is available on the dose-response relationship in humans for infection by *T. gondii*.

Appendix 2 contains detail on dose response.

No human dose-response information for *T. gondii* infection is available. A recent quantitative microbial risk assessment (QMRA) used dose-response information from mice as a surrogate for human dose-response (Opsteegh *et al.*, 2011a). The QMRA related to ingestion of the bradyzoites form of *T. gondii* from tissue cysts. The dose-response relationship is likely to be dependent on the form of the organism ingested, as well as the dose.

4.3 New Zealand Human Health Surveillance

KEY FINDINGS

The burden of toxoplasmosis in New Zealand can only be assessed through occasional seroprevalence studies and hospital discharges associated with congenital or acquired toxoplasmosis. The two seroprevalence studies reported since the last version of this Risk Profile give very similar estimates of seroprevalence in the adult population. These estimates are within the range of seroprevalence estimates found in earlier New Zealand studies.

4.3.1 *T. gondii* infection in New Zealand

Analysis of sera for infection determines the presence of IgG or IgM classes of antibodies specific for a *T. gondii* antigen. The presence of IgG antibodies (which persist in the body for long periods) indicates previous infection. IgM antibodies are a more recently available test, and this class of antibody rises early in infection. For infections with most other pathogens, IgM disappears in the weeks following primary infection. However, screening for toxoplasmosis is unusually difficult because of the persistence of the IgM antibody response. After primary *T. gondii* infection, IgM antibody frequently remains positive for many months or even years and may falsely indicate a recent infection (Morris and Croxson, 2004).

In some cases, antibody presence testing may be supplemented with antibody avidity testing. The avidity of the antibody-antigen reaction increases with time and low avidity is indicative of a recent infection (Hedman *et al.*, 1989; Holliman *et al.*, 1994)

Two small seroprevalence studies for *T. gondii* antibodies have been carried out in New Zealand since the previous version of this Risk Profile was published.

Healthy blood donors (n = 140) from the Waikato region were enrolled in a serology study during January and February 2005 (Zarkovic *et al.*, 2007). *T. gondii* IgG antibody titres were determined using a latex agglutination test and a titre cut-off of 1:32. Seropositivity for *T. gondii* was determined in 60/140 (42.9%, 95th percentile confidence interval 34.5-51.5%) samples. Seropositive donors were significantly older (mean 45 years) than seronegative donors (mean 40 years). Dog owners were statistically significantly more likely to be seropositive than cat owners.

A serological survey of staff at the Auckland Zoological Park was carried out in 1991 (n = 49), 2002 (n = 42) and 2010 (n = 46) for a range of potentially zoonotic infections (Forsyth *et al.*, 2012). *T. gondii* testing varied across the three years of the survey. In 1991, only IgM was tested, while in 2002 both IgM and IgG were tested. In 2010, all participants were tested for IgG antibodies, with those with levels greater than 20 IU/ml further tested for IgM and IgG avidity. In 2002 and 2010, 43% of participants were positive for *T. gondii* IgG antibodies.

However, few participants tested positive for IgM antibodies in any year and only one (in 2010) had IgM and low-avidity antibody, indicating infection in the previous 6-12 weeks. In 2010, of 19 IgG-positive zoo staff, 10 were domestic cat owners.

A study carried out in Auckland identified medical records from all cases who had a blood test for *T. gondii* IgG and IgM antibodies between 1 January and 30 November 2011 (IgG results were not reported) (Wong *et al.*, 2013). Of 1817 adult patients, 187 had a positive test for IgM antibodies at a concentration that suggested acute toxoplasmosis. Questionnaires were sent to 108 patients and were returned by 37. This group included 31 cases who had a positive test for IgM and reported lymphadenopathy. These cases were considered to have had acute toxoplasmosis. One case reported an immunosuppressive illness, while two were pregnant at the onset of acute toxoplasmosis. Risk factors reported by acute toxoplasmosis cases included, contact with cats or cat faeces (n = 16) and gardening with bare hands at least once per week (n = 4). However, a full list of the risk factors investigated was not reported. In addition to lymphadenopathy, fatigue, headaches, difficulty concentrating and muscle aches were symptoms reported by the majority of cases.

4.3.2 Toxoplasmosis

Two systematic sources of data are available for assessing the burden of clinical toxoplasmosis in New Zealand:

- Records of discharges from publically-funded hospitals[10]
- Records of mortality[11]

Records are coded for causative diseases using the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) developed by the World Health Organisation (WHO). The following ICD-10 codes relate to toxoplasmosis:

B 58 Toxoplasmosis (excludes congenital toxoplasmosis)
- B58.0 Toxoplasma oculopathy
- B58.1 Toxoplasma hepatitis
- B58.2 Toxoplasma meningoencephalitis
- B58.3 Pulmonary toxoplasmosis
- B58.8 Toxoplasmosis with other organ involvement (myocarditis, myositis)
- B58.9 Toxoplasmosis unspecified

P 37 Other congenital infectious and parasitic diseases
- P37.1 Congenital toxoplasmosis due to hydrocephalus

4.3.2.1 Mortality

In the period 2007-2011, one death from toxoplasmosis (B58) was reported. The fatality was a male aged 35-40 years.

[10] http://www.health.govt.nz/nz-health-statistics/health-statistics-and-data-sets/hospital-event-data-and-stats Accessed 27 February 2015

[11] http://www.health.govt.nz/nz-health-statistics/health-statistics-and-data-sets/mortality-data-and-stats Accessed 27 February 2015

4.3.2.2 Morbidity

Table 3 shows the number of cases discharged from public hospitals from 2007 to 2014 with a primary diagnosis of toxoplasmosis and a breakdown of the different clinical manifestations in terms of ICD-10 code (Chris Lewis, Ministry of Health, personal communication, 2015). Readmissions of the same patient have been excluded.

Table 3: **Public hospital discharge records for other acquired toxoplasmosis manifestations by year, gender and age range**

ICD-10 code	Year	Number of cases		Age range
		Male	Female	
B58.0 Toxoplasma oculopathy	2007	4	2	23-52
	2008	1	1	61-76
	2009	3	3	5-70
	2010	3	6	7-76
	2011	5	3	17-67
	2012	0	2	40-42
	2013	1	4	52-86
	2014	2	1	20-65
Total: 41 (26 in period 2001-2006)		**19**	**22**	
B58.2 Toxoplasma meningoencepthalitis	2007	1	1	42-56
	2008	2	1	28-47
	2009	1	0	42
	2010	3	1	35-52
	2011	1	1	42-56
	2012	1	0	29
	2013	2	0	51-53
	2014	1	1	31-50
Total: 15 (4 in period 2001-2006)		**11**	**4**	
B58.3 Pulmonary toxoplasmosis	2007-2014	0	0	
Total: 0 (4 in period 2001-2006)		**0**	**0**	
B58.8 Toxoplasmosis with other organ involvement (myocarditis, myositis)	2008	1	0	32
	2009	2	0	29-38
	2011	0	1	36
	2012	1	1	19-50
	2014	0	1	52
Total: 7 (13 in period 2001-2006)		**4**	**3**	
B58.9 Toxoplasmosis unspecified	2007	0	3	40-64

ICD-10 code	Year	Number of cases		Age range
		Male	Female	
	2008	3	1	36-47
	2009	0	2	53-56
	2010	2	1	13-44
	2011	3	3	26-53
	2012	4	3	9-50
	2013	1	2	6-49
	2014	0	1	24
Total: 26 (37 in period 2001-2006)		**13**	**13**	
Total for B58: 89 (84 in period 2001-2006)		**47**	**42**	

The total number of toxoplasmosis cases discharged from public hospitals per year, during the period 2007-2014 (11.1) was very similar to the discharge rate for the previous seven year period (2000-2006, 12 cases per year). However, a greater proportion of the cases during the period 2007-2014 had primary diagnoses of oculopathy and meningoencephalitis than in the earlier period.

During the period 2007-2014, 12 cases were discharged from public hospitals with a primary diagnosis of congenital toxoplasmosis (ICD-10 code P37.1) (Chris Lewis, Ministry of Health, personal communication, 2015). Of the 12 cases, 10 were less than one year of age. In comparison, 7 cases with a primary diagnosis of congenital toxoplasmosis were discharged during the period 2000-2006.

While the case numbers reported here are generally small, there is no indication that morbidity due to toxoplasmosis in New Zealand is decreasing with time.

4.3.3 Reported outbreaks

No outbreaks of toxoplasmosis have been reported in New Zealand.

4.3.4 Serotypes

Routine typing of *T. gondii* isolates is not currently carried out in New Zealand.

4.4 *T. gondii* Infection Overseas

KEY FINDINGS

The rate of *T. gondii* infection in New Zealand seroprevalence studies is within the range of studies reported from overseas. It should be noted that seroprevalence may vary significantly between countries and between different studies in the same country. There is evidence from some developed countries (e.g. France and USA) that the rate of *T. gondii* infection is decreasing over time. It has been suggested that this may be due to improved education of animal and human health practitioners and the public.

Appendix 2 contains detailed data summarised in this section.

Seroprevalence studies for *T. gondii* infection in humans are summarised in Appendix 2, Table 7.

Case control studies conducted overseas since 2006 and which examined meat consumption as a risk factor have been summarised in Appendix 2, Section 9.2.5. Meat or undercooked meat consumption was a significant risk factor in three of the five studies. A review of 14 older (pre-2006) case-control studies has pointed out that the attributable risks in such studies often do not explain the majority of cases (Petersen *et al.*, 2010). The paper also commented that cultural and eating habits can affect the epidemiology of the disease in different countries.

5 EVALUATION OF RISK

5.1 Existing Risk Assessments

KEY FINDINGS

A quantitative risk assessment for foodborne toxoplasmosis in New Zealand is not available. The only international, published risk assessment overestimates the incidence of disease compared to health records. Attribution of toxoplasmosis to foodborne transmission in several countries has been estimated to be in the range 30-50%.

Appendix 2 contains detailed data summarised in this section.

5.1.1 New Zealand risk assessment

No quantitative risk assessments for *T. gondii* in red meat and red meat products have been conducted in New Zealand.

5.1.2 Risk assessments from other countries

Risk assessments and risk ranking exercises are summarised in Appendix 2 Section 9.3. The single published quantitative risk assessment for *T. gondii* is from the Netherlands. Despite conservative assumptions regarding prevalence of meat contamination, this assessment overestimated incidence of illness when compared to human health records.

Burden of disease and attribution estimates for toxoplasmosis have been published for a number of countries (see Appendix 2, Section 9.4). Foodborne transmission of toxoplasmosis has been estimated for several countries as 30-50%. Estimates of disease burden for foodborne enteric pathogens have ranked foodborne toxoplasmosis as first (Netherlands), second (United States) and 11th (Canada).

5.2 Evaluation of Risk for New Zealand

KEY FINDINGS

The information on *T. gondii* in red meat and meat products in New Zealand since the previous Risk Profile is limited, and essentially the conclusions in the previous document still apply.

Expert opinion in New Zealand attributes 30% of transmission to foodborne routes. Apart from meat, fruits and vegetables, unpasteurised milk and possibly shellfish, are potential transmission vehicles. Given that infective cysts are readily destroyed by cooking and freezing, exposure from red meat appears unlikely to be the major foodborne transmission pathway.

There remain considerable methodological barriers to evaluating the risk of *T. gondii*, such as determining the infectivity status of contamination in meat and determining the prevalence and burden of disease. We consider that further work is justified on the risk from *T. gondii* in red meat, but initially the need is to characterise the burden of toxoplasmosis in New Zealand.

5.2.1 Risk associated with red meat and meat products

The 2008 Risk Profile concluded that:

- There is very little information on which to comment on the prevalence of clinical consequences of toxoplasmosis infection in New Zealand.
- The available data on seropositivity of the whole New Zealand population suggest that infection with *T. gondii* is as prevalent in New Zealand as other developed countries.
- It is extremely difficult to ascertain modes of transmission and, in particular, the extent of infection via the consumption of cysts in red meat.
- New Zealand has had no reported outbreaks of toxoplasmosis, and there have also been no case control studies, cohort studies or other risk factor studies. No data could be found on the prevalence of cysts in red meat or meat products in New Zealand.
- While information on seropositivity in New Zealand livestock is limited, it is likely that domestically-produced red meat in New Zealand does contain *T. gondii*. Similarly, imported red meat is likely to be infected with *T. gondii*, but will contribute less to total consumer exposure given that only relatively small amounts of meat are imported, and much of it is frozen. Ameliorating factors for any exposure are that animal seropositivity appears to overestimate infectivity, and *T. gondii* exposure will be controlled through cooking and freezing.

The information on *T. gondii* in red meat and meat products in New Zealand since the previous Risk Profile is limited, and essentially these conclusions still apply. It should be noted, that since the previous version of this Risk Profile the requirement for imported pigmeat to be frozen has been removed.[12]

The limited data on seroprevalence in New Zealand livestock indicates that infection is widespread, at least in deer, sheep and goats. The prevalence in New Zealand cattle is unknown, but the situation is unlikely to be markedly different to overseas where seroprevalence in cattle is lower than for other species, irrespectively of whether cattle are intensively or extensively reared. The widespread use of a vaccine in breeding ewes will reduce the risk from sheep meat (see Section 6.1.1). It is also clear that seroprevalence data are markedly higher than tissue infectivity determined by bioassay. Nevertheless, given the inability of meat inspection to exclude infected livestock at slaughter (see below), it is reasonable to expect a proportion of the red meat supply in New Zealand to contain infective cysts.

Expert opinion in New Zealand attributes approximately 30% of transmission to foodborne routes (Cressey and Lake, 2013). However, it should be noted that experts participating in the attribution study self-rating of their expertise with respect to *T. gondii* was the lowest of any of the nine pathogens considered. Apart from meat, fruits and vegetables, unpasteurised milk and possibly shellfish, are potential transmission vehicles. Given that infective cysts are readily destroyed by cooking and freezing, exposure from red meat appears unlikely to be the major foodborne transmission pathway.

The prevalence of *T. gondii* infection in the New Zealand population is unclear. A worldwide review of seroprevalence concluded that the prevalence in New Zealand (20-40%) was similar

[12] http://mpi.govt.nz/law-and-policy/requirements/import-health-standards/ Accessed 29 June 2015

to Australia, Chile and some parts of Europe, Africa, the Middle East and India (Pappas *et al.*, 2009). No consideration was given to the analytical methods used to determine seroprevalence.

The available morbidity and mortality data from hospital patients is well below estimates that can be derived from overseas developed country studies (see Section 5.3.2). There are some studies supporting under-recognition of infection. A study in Auckland published in 2004 found prevalences of IgG and IgM in blood samples from pregnant women of 33% and 2.4% respectively (Morris and Croxson, 2004). These results were used by the study authors to estimate that 296 of 14530 pregnant women in Auckland in the year 2000 would have had a positive IgM blood result, indicative of current or recent *T. gondii* infection. Based on an estimate of 10% for transmission of *T. gondii* infection to the foetus, this suggests potentially 30 congenital infections in the Auckland region per annum. More recent studies of immunocompetent patients in Auckland found a prevalence of IgM of 10% in cases for which samples were referred for *T. gondii* testing (Wong *et al.*, 2013).

The total number of toxoplasmosis cases discharged from public hospitals per year, during the period 2007-2014 was very similar to the discharge rate for the previous seven year period, suggesting that morbidity due to toxoplasmosis in New Zealand is not decreasing.

There remain considerable methodological barriers to evaluating the risk of *T. gondii*, such as determining the infectivity status of contamination in meat and determining the prevalence and burden of disease. We consider that further work is justified on the risk from *T. gondii* in red meat, but initially the need is to characterise the burden of toxoplasmosis in New Zealand.

5.2.2 Risks associated with other foods

Raw fruit and vegetable consumption has been identified as a risk factor in a number of seroprevalence studies, while unpasteurised milk and shellfish have been less frequently identified in these studies (see Table 7, Appendix 2). A recent case-control study in the USA identified consumption of raw beef, raw lamb, locally produced meat products, unpasteurised goats' milk and shellfish as risk factors (Jones *et al.*, 2009).

The prevalence of viable *T. gondii* in chickens from indoor farms has been reported to be low, while in backyard chickens and chickens from free-range farms the prevalence is up to 100% (Dubey, 2010). The characteristics of the range where backyard or free-range chickens feed may have an impact on the prevalence of infection. For example, the risk of infection may be higher if cats have access to the range. However, no studies were found that had investigated this possibility. *T. gondii* infection in chickens may present a risk of human infection if care is not taken with hand washing after handling chicken meat or if adequate cooking measures are not followed.

Waterborne toxoplasmosis is attracting increasing attention, following outbreaks linked to drinking water from contaminated reservoirs (Jones and Dubey, 2010).

5.3 The Burden of *T. gondii* Infection in New Zealand

KEY FINDINGS

There is no specific information on the burden of disease due to *T. gondii* infections in New Zealand. However, the available fragmentary evidence does not suggest that the situation with respect to *T. gondii* infections in New Zealand would be radically different to other developed countries, where toxoplasmosis has been estimated to be the major foodborne parasitic disease.

5.3.1 Burden of disease from red meat and meat products contaminated with *T. gondii*

It has been estimated by expert consultation that 27.6% (95th percentile credible interval 3.8-57.1%) of toxoplasmosis incidence is due to foodborne transmission (Cressey and Lake, 2013). An earlier expert elicitation derived a similar estimate of the proportion of toxoplasmosis cases that are believed to be due to transmission by food (31.5%) (Cressey and Lake, 2005). The earlier expert elicitation also derived an estimate for the proportion foodborne toxoplasmosis cases believed to be due to transmission by red meat (54.1%).

The 2013 expert elicitation also asked participants to self-rate their expertise with respect to each of nine pathogens considered. The average expertise assessment for *T. gondii* was the lowest of any of the nine pathogens considered.

Further elucidation of the burden of disease due to *T. gondii* in red meat and meat products in New Zealand is not possible due to the lack of any testing for *T. gondii* in foods. It is unlikely that *T. gondii* would be implicated as the cause of cases or outbreaks of foodborne disease in New Zealand.

5.3.2 Burden of disease from all *T. gondii* infections

There is no specific information on the burden of disease due to *T. gondii* infections in New Zealand. However, the available fragmentary evidence does not suggest that the situation with respect to *T. gondii* infections in New Zealand would be radically different to other developed countries, where toxoplasmosis has been estimated to be the major foodborne parasitic disease (see Appendix 2, section 9.4).

The recent study of Wong *et al*. (2012) suggests that cases of acute toxoplasmosis occur in New Zealand that would not be detected by existing surveillance systems.

The incidence of congenital toxoplasmosis in New Zealand is unknown. A systematic review of the worldwide incidence of congenital toxoplasmosis estimated that the incidence in the western Pacific region, including New Zealand, was 0.6 cases per 1000 live births (95th percentile confidence interval 0.5-0.8) (Torgerson and Mastroiacovo, 2013). Using this estimate, and with 57,242 live births in New Zealand during the 2014 year[13], approximately 30-50 cases of congenital toxoplasmosis may be expected in New Zealand each year. However,

[13] http://www.stats.govt.nz/browse_for_stats/population/births/BirthsAndDeaths_HOTPYeDec14.aspx Accessed 18 March 2015

hospital discharge data (section 4.3.2.2) suggests that no more than two congenital toxoplasmosis cases per year occur in New Zealand.

5.4 Data Gaps

KEY FINDINGS

Little progress has been made on addressing previous identified data gaps, to determine the prevalence and concentration of *T. gondii* contamination in red meat and meat products and the incidence of toxoplasmosis in New Zealand.

The data gaps identified in the 2008 Risk Profile (Lake *et al.*, 2008) and updated commentary on these are presented in Table 4.

A newly identified data gap is:

- Genotyping of New Zealand *T. gondii* strains to determine if they are more akin to the largely clonal populations seen in Europe and North America or the more diverse population seen in South America.

Table 4: Data gaps identified in the 2008 Risk Profile

Data gap	Commentary
Prevalence of *T. gondii* infection in livestock in New Zealand	Seroprevalence study in sheep published (Dempster *et al.*, 2011)
Prevalence of contamination of red meat and red meat products with *T. gondii* in New Zealand	No new information
Accurate data concerning toxoplasmosis cases in at risk groups	No new information
Lack of data regarding prevalence of infection in domestic cats (the definitive host) in New Zealand	No new information
Information on awareness by at risk groups in relation to the transmission risks of *T. gondii*. This information would be useful in targeting risk communication messages	While information on *T. gondii* infections are available to at-risk groups, no information on awareness amongst these groups was found.

6 AVAILABILITY OF CONTROL MEASURES

6.1 Current Control Measures

KEY FINDINGS

A vaccine to prevent *T. gondii* infection in breeding ewes is widely used in New Zealand to prevent lamb abortions. Meat inspection at slaughter is unlikely to identify infected carcasses. Cooking and freezing are effective control steps.

6.1.1 Biocontrols

Infections due to *T. gondii*, *Campylobacter fetus fetus* and *Salmonella* Brandenburg have been reported to cause more than 80% of ovine abortions in New Zealand (West, 2002). *T. gondii* vaccines are available in New Zealand for use on sheep (Toxovax®), primarily to prevent abortions.[14]. However, there is also evidence that this vaccine may inhibit formation of *T. gondii* tissue cysts (Innes *et al.*, 2009; Katzer *et al.*, 2014). This vaccine, developed in New Zealand, has been marketed since 1988. The vaccine is a live suspension of an attenuated form of the S48 strain tachyzoites and is estimated to be used on 80% of the breeding ewe population in New Zealand (Sam Higgins, MSD Animal Health, Wellington, personal communication, April 2015).

6.1.2 Carcass inspection

Inspection of bovine carcasses during meat processing in New Zealand for the presence of *T. gondii* is evaluated in a publication by the Ministry for Primary Industries (MPI, 2011). The review comments, "Lesions in lymph nodes are often associated with pathology of the corresponding organ. They are characterised by irregular areas of coagulative necrosis, mainly in the cortex and a moderate inflammatory reaction may be evident at the periphery of the necrotic areas. However, such pathological changes are unremarkable when lymph nodes are grossly examined; are not prominent visually and are unlikely to be seen by routine post mortem examination."

The overall conclusion is: "Due to the limited and unremarkable pathology associated with occasional infection of cattle with *Toxoplasma* spp., routine post mortem inspection of lymph nodes is highly unlikely to identify infected animals. Further, the public health benefit of identification of such infections is unknown."

The MPI document also comments that cattle are also not believed to remain persistently infected as long as sheep, and epidemiological studies point to consumption of raw or undercooked mutton and pork as the most important risk factors for human transmission during pregnancy. The case control studies summarised in Appendix 2, Section 9.2.5 provide mixed evidence for this comment, in that a study in the USA found that lamb had higher risk than beef, while a study in Serbia found that beef had higher risk than other meat types. However, the seroprevalence data collated in Appendix 1, Table 4 do generally support this statement.

[14] http://www.msd-animal-health.co.nz/Products/Toxovax/020_Product-Overview.aspx Accessed 10 March 2015

6.1.3 Food processing

Cooking and freezing are important control steps. The previous Risk Profile summarised information showing that cysts are inactivated essentially immediately at temperatures above 67°C (D time 1 second at 67°C (Dubey *et al.*, 1990). Microwave cooking appears to be less effective, possibly due to uneven heating. New information in this update (Section 2.3.2) largely supports these parameters, although the study of El-Nawawi (2008) suggests greater thermal stability of *T. gondii* cysts in sheep meat, with infectivity remaining after heating at 60°C or 100°C for 5 minutes, but not after 10 minutes heating. These studies suggest that further work may be required before definitive guidelines on thermal inactivation of *T. gondii* can be made.

Freezing has been reported to be effective in inactivating *T. gondii* cysts (El-Nawawi *et al.*, 2008; Gencay *et al.*, 2013; Neumayerová *et al.*, 2014). However, there is some variation in reported times to inactivation, with cysts in goat meat reported to be inactivated after 4 hours at -20°C (Neumayerová *et al.*, 2014), while cysts in sheep meat were reported to be still infective after 1 day at -20°C, but not after 2 days (El-Nawawi *et al.*, 2008). If all available studies are considered, it appears likely that *T. gondii* cysts will be inactivated by freezing at -18 to -20°C for 2 days.

There are some data to support control by processing (salt, pH, nitrite) but these would need to be validated for specific processes.

6.1.4 Consumer education

Education of at-risk groups is considered to be an important control measure for *T. gondii* infection.

6.1.4.1 Pregnant women

A publication from the Ministry for Primary Industries (MPI), entitled "Food Safety in Pregnancy"[15], highlights risk due to toxoplasmosis and identifies contact with cats and "eating unwashed vegetables, undercooked meat, or drinking raw or unpasteurised milk and ready-to-eat cured meats such as salami and ham" as risk factors. Further information is available in a report prepared for the New Zealand Food Safety Authority (now incorporated into MPI) by ESR (Gilbert *et al.*, 2010).

The Ministry of Health (MoH) also include information and advice related to toxoplasmosis in their "Food and Nutrition Guidelines for Healthy Pregnant and Breastfeeding Women" (Ministry of Health, 2008). Specifically, this publication states that "Infection is acquired by eating food or water contaminated with oocysts shed by cats, or by eating raw or under-cooked contaminated meat (mainly pork or lamb) containing tissue cysts, or by drinking water or food contaminated with oocysts from the faeces of infected cats".

[15] http://www.foodsmart.govt.nz/elibrary/consumer/2013-mpi-food-safety-in-pregnancy-web.pdf Accessed 10 March 2015

6.1.4.2 People with HIV/AIDS

MoH provide information for people with HIV/AIDS, including information on opportunistic infections.[16] The information on opportunistic infections includes reference to a document produced in the USA by the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America (Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents, 2014). This document includes the information "Primary infection occurs after eating undercooked meat containing tissue cysts or ingesting oocysts that have been shed in cat feces and sporulated in the environment, a process that takes at least 24 hours. In the USA, eating raw shellfish including oysters, clams, and mussels recently was identified as a novel risk factor for acute infection. Up to 50% of individuals with documented primary infection do not have an identifiable risk factor. The organism is not transmitted through person-to-person contact".

[16] http://www.health.govt.nz/our-work/diseases-and-conditions/hiv-and-aids/hiv-aids-research-and-information Accessed 10 March 2015

7 REFERENCES

Abdulmawjood A, Rosa S, Taubert A, Bauer C, Failing K, Zahner H, Bülte M. (2014) Investigation of persistence of infectious *Toxoplasma gondii* in raw sausages using in-house developed and validated real time-PCR. Meat Science; 97(4): 542-547.

Abu EK, Boampong JN, Ayi I, Ghartey-Kwansah G, Afoakwah R, Nsiah P, Blay E. (2014) Infection risk factors associated with seropositivity for *Toxoplasma gondii* in a population-based study in the Central Region, Ghana. Epidemiology and Infection; FirstView: 1-9.

Ahmad N, Qayyum M. (2014) Seroprevalence and risk factors for toxoplasmosis in large ruminants in northern Punjab, Pakistan. Journal of Infection in Developing Countries; 8(8): 1022-1028.

Ahn H-J, Cho PY, Ahn SK, Kim T-S, Chong C-K, Hong S-J, Cha SH, Nam H-W. (2012) Seroprevalence of toxoplasmosis in the residents of Cheorwon-gun, Gangwon-do, Korea. Korean Journal of Parasitology; 50(3): 225-227.

Akanmu AS, Osunkalu VO, Ofomah JN, Olowoselu FO. (2010) Pattern of demographic risk factors in the seroprevalence of anti-*Toxoplasma gondii* antibodies in HIV infected patients at the Lagos University Teaching Hospital. Nigerian Quarterly Journal of Hospital Medicine; 20(1): 1-4.

Al Mohammad HI, Amin TT, Balaha MH, Al Moghannum MS. (2010) Toxoplasmosis among the pregnant women attending a Saudi maternity hospital: seroprevalence and possible risk factors. Annals of Tropical Medicine and Parasitology; 104(6): 493-504.

Alanazi AD. (2013) Determination of seropositivity for *Toxoplasma gondii* in sheep, goats and camels slaughtered for food and human consumptions in Riyadh municipal abattoirs, Saudi Arabia. Journal of the Egyptian Society of Parasitology; 43(3): 569-576.

Albuquerque GR, Munhoz AD, Teixeira M, Flausino W, de Medeiros SM, Lopes CWG. (2011) Risk factors associated with *Toxoplasma gondii* infection in dairy cattle, State of Rio de Janeiro. Pesquisa Veterinaria Brasileira; 31(4): 287-290.

Aldebert D, Hypolite M, Cavailles P, Touquet B, Flori P, Loeuillet C, Cesbron-Delauw MF. (2011) Development of high-throughput methods to quantify cysts of *Toxoplasma gondii*. Cytometry Part A; 79A(11): 952-958.

Alvarado-Esquivel C, Alanis-Quiñones OP, Arreola-Valenzuela MA, Rodríguez-Briones A, Piedra-Nevarez LJ, Duran-Morales E, Estrada-Martínez S, Martínez-García SA, Liesenfeld O. (2006) Seroepidemiology of *Toxoplasma gondii* infection in psychiatric inpatients in a northern Mexican city. BMC Infectious Diseases; 6: 178.

Alvarado-Esquivel C, Mercado-Suarez MF, Rodriguez-Briones A, Fallad-Torres L, Ayala-Ayala JO, Nevarez-Piedra LJ, Duran-Morales E, Estrada-Martinez S, Liesenfeld O, Marquez-Conde JA, Martinez-Garcia SA. (2007) Seroepidemiology of infection with *Toxoplasma gondii* in healthy blood donors of Durango, Mexico. BMC Infectious Diseases; 7: 75.

Alvarado-Esquivel C, Cruz-Magallanes HM, Esquivel-Cruz R, Estrada-Martínez S, Rivas-González M, Liesenfeld O, Martínez-García SA, Ramírez E, Torres-Castorena A, Castañeda A, Dubey JP. (2008) Seroepidemiology of *Toxoplasma gondii i*nfection in human adults from three rural communities in Durango State, Mexico. Journal of Parasitology; 94(4): 811-816.

Alvarado-Esquivel C, Liesenfeld O, Torres-Castorena A, Estrada-Martinez S, Urbina-Alvarez JD, Ramos-de la Rocha M, Marquez-Conde JA, Dubey JP. (2010a) Seroepidemiology of *Toxoplasma gondii* infection in patients with vision and hearing impairments, cancer, HIV, or undergoing hemodialysis in Durango, Mexico. Journal of Parasitology; 96(3): 505-508.

Alvarado-Esquivel C, Rojas-Rivera A, Estrada-Martinez S, Sifuentes-Alvarez A, Liesenfeld O, Garcia-Lopez CR, Dubey JP. (2010b) Seroepidemiology of *Toxoplasma gondii* infection in a Mennonite community in Durango state, Mexico. Journal of Parasitology; 96(5): 941-945.

Alvarado-Esquivel C, Estrada-Martinez S, Liesenfeld O. (2011a) *Toxoplasma gondii* infection in workers occupationally exposed to unwashed raw fruits and vegetables: a case control seroprevalence study. Parasites and Vectors; 4: 235.

Alvarado-Esquivel C, Estrada-Martinez S, Pizarro-Villalobos H, Arce-Quinones M, Liesenfeld O, Dubey JP. (2011b) Seroepidemiology of *Toxoplasma gondii* infection in general population in a northern Mexican city. Journal of Parasitology; 97(1): 40-43.

Alvarado-Esquivel C, Garcia-Machado C, Alvarado-Esquivel D, Gonzalez-Salazar AM, Briones-Fraire C, Vitela-Corrales J, Villena I, Dubey JP. (2011c) Seroprevalence of *Toxoplasma gondii* infection in domestic pigs in Durango State, Mexico. Journal of Parasitology; 97(4): 616-619.

Alvarado-Esquivel C, Liesenfeld O, Estrada-Martínez S, Félix-Huerta J. (2011d) *Toxoplasma gondii* infection in workers occupationally exposed to raw meat. Occupational Medicine; 61(4): 265-269.

Alvarado-Esquivel C, Torres-Berumen JL, Estrada-Martínez S, Liesenfeld O, Mercado-Suarez MF. (2011e) *Toxoplasma gondii* infection and liver disease: A case-control study in a Northern Mexican population. Parasites and Vectors; 4(1): 75.

Alvarado-Esquivel C, Estrada-Malacon MA, Reyes-Hernandez SO, Perez-Ramirez JA, Trujillo-Lopez JI, Villena I, Dubey JP. (2012a) High prevalence of *Toxoplasma gondii* antibodies in domestic pigs in Oaxaca State, Mexico. Journal of Parasitology; 98(6): 1248-1250.

Alvarado-Esquivel C, García-Machado C, Alvarado-Esquivel D, Vitela-Corrales J, Villena I, Dubey JP. (2012b) Seroprevalence of *Toxoplasma gondii* infection in domestic sheep in Durango State, Mexico. Journal of Parasitology; 98(2): 271-273.

Alvarado-Esquivel C, Liesenfeld O, Burciaga-Lopez BD, Ramos-Nevarez A, Estrada-Martinez S, Cerrillo-Soto SM, Carrete-Ramirez FA, Lopez-Centeno Mde L, Ruiz-Martinez MM. (2012c) Seroepidemiology of *Toxoplasma gondii* infection in elderly people in a northern Mexican city. Vector Borne and Zoonotic Diseases; 12(7): 568-574.

Alvarado-Esquivel C, Torres-Castorena A, Liesenfeld O, Estrada-Martinez S, Urbina-Alvarez JD. (2012d) High seroprevalence of *Toxoplasma gondii* infection in a subset of Mexican patients with work accidents and low socioeconomic status. Parasites and Vectors; 5: 15.

Alvarado-Esquivel C, Campillo-Ruiz F, Liesenfeld O. (2013a) Seroepidemiology of infection with *Toxoplasma gondii* in migrant agricultural workers living in poverty in Durango, Mexico. Parasites and Vectors; 6: 113.

Alvarado-Esquivel C, Estrada-Malacón MA, Reyes-Hernández SO, Pérez-Ramírez JA, Trujillo-López JI, Villena I, Dubey JP. (2013b) Seroprevalence of *Toxoplasma gondii* in domestic sheep in Oaxaca State, Mexico. Journal of Parasitology; 99(1): 151-152.

Alvarado-Esquivel C, Silva-Aguilar D, Villena I, Dubey JP. (2013c) Seroprevalence of *Toxoplasma gondii* infection in dairy goats in Michoacán State, Mexico. Journal of Parasitology; 99(3): 540-542.

Alvarado-Esquivel C, Silva-Aguilar D, Villena I, Dubey JP. (2013d) Seroprevalence and correlates of *Toxoplasma gondii* infection in domestic sheep in Michoacán State, Mexico. Preventive Veterinary Medicine; 112(3–4): 433-437.

Alvarado-Esquivel C, Janet Pacheco-Vega S, Hernandez-Tinoco J, Francisco Sanchez-Anguiano L, Omar Berumen-Segovia L, Imard Rodriguez-Acevedo FJ, Beristain-Garcia I, Rabago-Sanchez E, Liesenfeld O, Campillo-Ruiz F, Alberto Guereca-Garcia O. (2014a) Seroprevalence of *Toxoplasma gondii* infection and associated risk factors in Huicholes in Mexico. Parasites and Vectors; 7: e301.

Alvarado-Esquivel C, Romero-Salas D, García-Vázquez Z, Crivelli-Diaz M, Barrientos-Morales M, Lopez-de-Buen L, Dubey J. (2014b) Seroprevalence and correlates of *Toxoplasma gondii* infection in domestic pigs in Veracruz State, Mexico. Tropical Animal Health and Production; 46(4): 705-709.

Anastasia D, Elias P, Nikolaos P, Charilaos K, Nektarios G. (2013) *Toxoplasma gondii* and *Neospora caninum* seroprevalence in dairy sheep and goats mixed stock farming. Veterinary Parasitology; 198(3–4): 387-390.

Andrade MMC, Carneiro M, Medeiros AD, Neto VA, Vitor RWA. (2013) Seroprevalence and risk factors associated with ovine toxoplasmosis in Northeast Brazil. Parasite; 20: 20.

APHIS. (2014) Toxoplasma on U.S. sheep operations. Accessed at: http://www.aphis.usda.gov/animal_health/nahms/sheep/downloads/sheep11/Sheep11_is_Toxo.pdf. Accessed: 16 December 2014.

Arias I, Sorlozano A, Villegas E, Luna JdD, McKenney K, Cervilla J, Gutierrez B, Gutierrez J. (2012) Infectious agents associated with schizophrenia: A meta-analysis. Schizophrenia Research; 136(1–3): 128-136.

Asgari Q, Mehrabani D, Moazeni M, Mohajeri FA, Kalantari M, Motazedian MH, Hatam GR, Davarpanah MA. (2010) The seroprevalence of bovine toxoplasmosis in Fars province, Southern Iran. Asian Journal of Animal and Veterinary Advances; 5(3): 210-216.

Asgari Q, Sarkari B, Amerinia M, Panahi S, Mohammadpour I, Sadeghi Sarvestani A. (2013) Toxoplasma infection in farm animals: A seroepidemiological survey in Fars Province, South of Iran. Jundishapur Journal of Microbiology; 6(3): 269-272.

Aspinall TV, Marlee D, Hyde JE, Sims PFG. (2002) Prevalence of *Toxoplasma gondii* in commercial meat products as monitored by polymerase chain reaction - Food for thought? International Journal for Parasitology; 32(9): 1193-1199.

Avelino MM, Campos Jr D, De Parada JDCB, De Castro AM. (2003) Pregnancy as a risk factor for acute toxoplasmosis seroconversion. European Journal of Obstetrics Gynecology and Reproductive Biology; 108(1): 19-24.

Azizi H, Shiran B, Boroujeni AB, Jafari M. (2014) Molecular survey of *Toxoplasma gondii* in sheep, cattle and meat products in Chaharmahal va Bakhtiari province, Southwest of Iran. Iranian Journal of Parasitology; 9(3): 429-434.

Bao M, Tian YM, Zhou DH, Xu MJ, Wu SM, Wang SD, Tang F, Qu LD, Song HQ. (2012) Seroprevalence of *Toxoplasma gondii* infection in dairy cattle, in Jinzhou, Northeastern China. African Journal of Microbiology Research; 6(17): 3932-3935.

Bártová E, Sedlák K, Literák I. (2009) *Toxoplasma gondii* and *Neospora caninum* antibodies in sheep in the Czech Republic. Veterinary Parasitology; 161(1–2): 131-132.

Bártová E, Sedlák K. (2011) Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in slaughtered pigs in the Czech Republic. Parasitology; 138(11): 1369-1371.

Basso W, Hartnack S, Pardini L, Maksimov P, Koudela B, Venturini MC, Schares G, Sidler X, Lewis FI, Deplazes P. (2013) Assessment of diagnostic accuracy of a commercial ELISA for the detection of *Toxoplasma gondii* infection in pigs compared with IFAT, TgSAG1-ELISA and Western blot, using a Bayesian latent class approach. International Journal for Parasitology; 43(7): 565-570.

Bayarri S, Gracia MJ, Lázaro R, Pe´rez-Arquillué C, Barberán M, Herrera A. (2010) Determination of the viability of *Toxoplasma gondii* in cured ham using bioassay: Influence of technological processing and food safety implications. Journal of Food Protection; 73(12): 2239-2243.

Bayarri S, Gracia MJ, Pérez-Arquillué C, Lázaro R, Herrera A. (2012) *Toxoplasma gondii* in commercially available pork meat and cured ham: A contribution to risk assessment for consumers. Journal of Food Protection; 75(3): 597-600.

Beef + Lamb New Zealand. (2014) Compendium of New Zealand farm facts 2014. Accessed at: http://www.beeflambnz.com/Documents/Information/Compendium%20of%20New%20Zealand%20farm%20facts.pdf. Accessed: 16 March 2015.

Belfort-Neto R, Nussenblatt V, Rizzo L, Muccioli C, Silveira C, Nussenblatt R, Khan A, Sibley LD, Belfort R, Jr. (2007) High prevalence of unusual genotypes of *Toxoplasma gondii* infection in pork meat samples from Erechim, Southern Brazil. Anais da Academia Brasileira de Ciencias; 79(1): 111-114.

Berger-Schoch AE, Bernet D, Doherr MG, Gottstein B, Frey CF. (2011a) *Toxoplasma gondii* in Switzerland: A serosurvey based on meat juice analysis of slaughtered pigs, wild boar, sheep and cattle. Zoonoses and Public Health; 58(7): 472-478.

Berger-Schoch AE, Herrmann DC, Schares G, Müller N, Bernet D, Gottstein B, Frey CF. (2011b) Prevalence and genotypes of *Toxoplasma gondii* in feline faeces (oocysts) and meat from sheep, cattle and pigs in Switzerland. Veterinary Parasitology; 177(3–4): 290-297.

Berrébi A, Assouline C, Bessières M-H, Lathière M, Cassaing S, Minville V, Ayoubi J-M. (2010) Long-term outcome of children with congenital toxoplasmosis. American Journal of Obstetrics and Gynecology; 203(6): 552.e551-552.e556.

Bezerra MJG, Cruz JALO, Kung ES, Silva JG, Santos AS, Moraes EPBX, Pinheiro Junior JW, Mota RA. (2014) Occurrence of *Toxoplasma gondii* DNA in sheep naturally infected and slaughtered in abattoirs in Pernambuco, Brazil. Pesquisa Veterinaria Brasileira; 34(4): 329-331.

Bezerra RA, Carvalho FS, Guimarães LA, Rocha DS, Maciel BM, Wenceslau AA, Lopes CWG, Albuquerque GR. (2012a) Genetic characterization of *Toxoplasma gondii* isolates from pigs intended for human consumption in Brazil. Veterinary Parasitology; 189(2–4): 153-161.

Bezerra RA, Carvalho FS, Guimarães LA, Rocha DS, Silva F, Wenceslau AA, Albuquerque GR. (2012b) Comparison of methods for detection of *Toxoplasma gondii* in tissues of naturally exposed pigs. Parasitology Research; 110(2): 509-514.

Blagojevic B, Antic D. (2014) Assessment of potential contribution of official meat inspection and abattoir process hygiene to biological safety assurance of final beef and pork carcasses. Food Control; 36(1): 174-182.

Bobic B, Nikolic A, Klun I, Djurkovic-Djakovic O. (2011) Kinetics of Toxoplasma infection in the Balkans. Wiener Klinische Wochenschrift; 123: 2-6.

Bobić B, Nikolić A, Klun I, Vujanić M, Djurković-Djaković O. (2007) Undercooked meat consumption remains the major risk factor for Toxoplasma infection in Serbia. Parassitologia; 49(4): 227-230.

Boughattas S, Ayari K, Sa T, Aoun K, Bouratbine A. (2014) Survey of the parasite *Toxoplasma gondii* in human consumed ovine meat in Tunis City. PLoS ONE; 9(1): e85044.

Bowie WR, King AS, Werker DH, IsaacRenton JL, Bell A, Eng SB, Marion SA. (1997) Outbreak of toxoplasmosis associated with municipal drinking water. Lancet; 350(9072): 173-177.

Boyer K, Hill D, Mui E, Wroblewski K, Karrison T, Dubey JP, Sautter M, Noble AG, Withers S, Swisher C, Heydemann P, Hosten T, Babiarz J, Lee D, Meier P, McLeod R. (2011) Unrecognized ingestion of *Toxoplasma gondii* oocysts leads to congenital toxoplasmosis and causes epidemics in North America. Clinical Infectious Diseases; 53(11): 1081-1089.

Brownback KR, Crosser MS, Simpson SQ. (2012) A 49-year-old man with chest pain and fever after returning from France. Chest; 141(6): 1618-1621.

Buery JC, Fux B, Vitor RWA, Sartori F, Cerutti Jr C. (2014) Analysis of seroconversion rate and factors associated with toxoplasmosis in a rural area of an extra-Amazonian region in Brazil: A cohort study. Open Tropical Medicine Journal; 7(1): 1-10.

Butler AJ, Thomas MK, Pintar KD. (2015) Expert Elicitation as a Means to Attribute 28 Enteric Pathogens to Foodborne, Waterborne, Animal Contact, and Person-to-Person Transmission Routes in Canada. Foodborne Pathog Dis; 12(4): 335-344.

Buxton D, Rodger SM, Maley SW, Wright SE. (2006) Toxoplasmosis: The possibility of vertical transmission. Small Ruminant Research; 62(1–2): 43-46.

Cademartori BG, Santos LMJF, Oliveira FC, Quevedo P, Oliveira PA, Ramos TS, Rocha ASR, Ruas JL, Farias NAR. (2014) Isolation and pathogenicity of *Toxoplasma gondii* in naturally infected (rustic farm) pigs in southern Brazil. Veterinary Parasitology; 203(1–2): 207-211.

Carellos EVM, De Andrade GMQ, Vasconcelos-Santos DV, Januário JN, Romanelli RMC, Abreu MNS, Da Silva FM, Loures IRC, De Andrade JQ, Caiaffa WT. (2014) Adverse socioeconomic conditions and oocyst-related factors are associated with congenital toxoplasmosis in a population-based study in Minas Gerais, Brazil. PLoS ONE; 9(2): e88588.

Carneiro ACAV, Carneiro M, Gouveia AMG, Vilas-Boas LS, Vitor RWA. (2009) Seroprevalence and risk factors of sheep toxoplasmosis in Minas Gérais, Brazil. Revue de Medecine Veterinaire; 160(11): 527-531.

Cavalcante ACR, Carneiro M, Gouveia AMG, Pinheiro RR, Vitor RWA. (2008) Risk factors for infection by *Toxoplasma gondii* in herds of goats in Ceara, Brazil. Arquivo Brasileiro De Medicina Veterinaria E Zootecnia; 60(1): 36-41.

Cenci-Goga BT, Ciampelli A, Sechi P, Veronesi F, Moretta I, Cambiotti V, Thompson PN. (2013) Seroprevalence and risk factors for *Toxoplasma gondii* in sheep in Grosseto district, Tuscany, Italy. BMC Veterinary Research; 9: 25.

Chandrawathani P, Nurulaini R, Zanin CM, Premaalatha B, Adnan M, Jamnah O, Khor SK, Khadijah S, Lai SZ, Shaik MA, Seah TC, Zatil SA. (2008) Seroprevalence of *Toxoplasma gondii* antibodies in pigs, goats, cattle, dogs and cats in peninsular Malaysia. Tropical Biomedicine; 25(3): 257-258.

Chang Q-C, Zheng X, Qiu J-H, Wang C-R, Zhu X-Q. (2012) Seroprevalence of *Toxoplasma gondii* infection in fattening pigs in Northeast China. Journal of Parasitology; 99(3): 544-545.

Chiang T-Y, Hsieh H-H, Kuo M-C, Chiu K-T, Lin W-C, Fan C-K, Fang C-T, Ji D-D. (2012) Seroepidemiology of *Toxoplasma gondii* infection among healthy blood donors in Taiwan. PLoS ONE; 7(10): e48139.

Chiang TY, Kuo MC, Chen CH, Yang JY, Kao CF, Ji DD, Fang CT. (2014) Risk factors for acute *Toxoplasma gondii* diseases in Taiwan: A population-based case-control study. PLoS ONE; 9(3): e90880.

Chikweto A, Kumthekar S, Tiwari K, Nyack B, Deokar MS, Stratton G, Macpherson CNL, Sharma RN, Dubey JP. (2011) Seroprevalence of *Toxoplasma gondii* in pigs, sheep, goats, and cattle from Grenada and Carriacou, West Indies. Journal of Parasitology; 97(5): 950-951.

Clementino MM, Souza MF, Neto VFA. (2007) Seroprevalence and *Toxoplasma gondii*-IgG avidity in sheep from Lajes, Brazil. Veterinary Parasitology; 146(3–4): 199-203.

Coelho L, Veloso VG, Grinsztejn B, Luz PM. (2014) Trends in overall opportunistic illnesses, *Pneumocystis carinii* pneumonia, cerebral toxoplasmosis and *Mycobacterium avium* complex incidence rates over the 30 years of the HIV epidemic: a systematic review. Brazilian Journal of Infectious Diseases; 18(2): 196-210.

Contopoulos-Ioannidis D, Talucod J, Maldonado Y, Montoya JG. (2014) Seasonal variation of acute toxoplasmic lymphadenopathy in the United States. Epidemiology and Infection; FirstView: 1-5.

Contopoulos-Ioannidis DG, Maldonado Y, Montoya JG. (2013) Acute *Toxoplasma gondii* infection among family members in the United States. Emerging Infectious Diseases; 19(12): 1981-1984.

Correa R, Cedeño I, de Escobar C, Fuentes I. (2008) Increased urban seroprevalence of *Toxoplasma gondii* infecting swine in Panama. Veterinary Parasitology; 153(1–2): 9-11.

Cosendey-KezenLeite RIJ, de Oliveira FCR, Frazão-Teixeira E, Dubey JP, de Souza GN, Ferreira AMR, Lilenbaum W. (2014) Occurrence and risk factors associated to *Toxoplasma gondii* infection in sheep from Rio de Janeiro, Brazil. Tropical Animal Health and Production; 46(8): 1463-1466.

Cressey P, Lake R. (2005) Ranking food safety risks. Development of NZFSA policy 2004-2005. ESR Client Report FW0563. Christchurch: ESR.

Cressey P, King N, Lake R. (2006) Food consumption data for risk assessments. ESR Client Report FW0602. Christchurch: ESR.

Cressey P. (2013) Food consumption data for risk assessments. ESR Client Report FW13008. Christchurch: ESR.

Cressey P, Lake R. (2013) Expert elicitation: Foodborne transmission of enteric pathogens in New Zealand. Client Report FW13032. Christchurch: ESR.

Cummings PL, Kuo T, Javanbakht M, Sorvillo F. (2014) Trends, productivity losses, and associated medical conditions among toxoplasmosis deaths in the United States, 2000–2010. American Journal of Tropical Medicine and Hygiene; 91(5): 959-964.

Dattoli VCC, Veiga RV, Cunha SS, Pontes-de-Carvalho L, Barreto ML, Alcantara-Neves NM. (2011) Oocyst ingestion as an important transmission route of *Toxoplasma gondii* in Brazilian urban children. Journal of Parasitology; 97(6): 1080-1084.

Daunter B. (1992) Immunology of pregnancy: Towards a unifying hypothesis. European Journal of Obstetrics Gynecology and Reproductive Biology; 43(2): 81-95.

Davoust B, Mediannikov O, Sambou M, Roqueplo C, Demoncheaux JP, Perret C, Guillot J, Blaga R. (2015) [Serological survey of animal toxoplasmosis in Senegal.]. Bulletin de la Societe de Pathologie Exotique; 108(1): 73-77.

de F. Santos LMJ, Damé MCF, Cademartori BG, da Cunha Filho NA, da R. Farias NA, Ruas JL. (2013) Occurrence of antibodies to *Toxoplasma gondii* in water buffaloes and meat cattle in Rio Grande do Sul State, southern Brazil. Acta Parasitologica; 58(3): 334-336.

de la Luz Galvan-Ramirez M, Troyo R, Roman S, Calvillo-Sanchez C, Bernal-Redondo R. (2012) A systematic review and meta-analysis of *Toxoplasma gondii* infection among the Mexican population. Parasites and Vectors; 5: 271.

de Moura AB, Osaki SC, Zulpo DL, Garcia JL, Teixeira EB. (2010) Detection of anti-*Toxoplasma gondii* antibodies in beef cattle slaughtered on Guarapuava city, Paraná State, Brazil. Archives of Veterinary Science; 15(2): 94-99.

de Sousa RA, Lemos Jda F, Farias LA, Lopes CD, dos Santos KR. (2014) Seroprevalence and risk factors for *Toxoplasma gondii* infection in pigs in southern Piaui. Revista Brasileira De Parasitologia Veterinaria; 23(1): 98-100.

Deksne G, Kirjusina M. (2013) Seroprevalence of *Toxoplasma gondii* in domestic pigs (*Sus scrofa domestica*) and wild boars (*Sus scrofa*) in Latvia. Journal of Parasitology; 99(1): 44-47.

Demar M, Ajzenberg D, Maubon D, Djossou F, Panchoe D, Punwasi W, Valery N, Peneau C, Daigre J-L, Aznar C, Cottrelle B, Terzan L, Dardé M-L, Carme B. (2007) Fatal outbreak of human toxoplasmosis along the Maroni River: Epidemiological, clinical, and parasitological aspects. Clinical Infectious Diseases; 45(7): e88-e95.

Dempster RP, Wilkins M, Green RS, de Lisle GW. (2011) Serological survey of *Toxoplasma gondii* and *Campylobacter fetus fetus* in sheep from New Zealand. New Zealand Veterinary Journal; 59(4): 155-159.

Derakhshan M, Mousavi M. (2014) Serological survey of antibodies to *Toxoplasma gondii* in cats, goats, and sheep in Kerman, Iran. Comparative Clinical Pathology; 23(2): 267-268.

Devleesschauwer B, Pruvot M, Joshi DD, De Craeye S, Jennes M, Ale A, Welinski A, Lama S, Aryal A, Victor B, Duchateau L, Speybroeck N, Vercruysse J, Dorny P. (2013) Seroprevalence of zoonotic parasites in pigs slaughtered in the Kathmandu Valley of Nepal. Vector-Borne and Zoonotic Diseases; 13(12): 872-876.

Devleesschauwer B, Ale A, Torgerson P, Praet N, de Noordhout CM, Pandey BD, Pun SB, Lake R, Vercruysse J, Joshi DD, Havelaar AH, Duchateau L, Dorny P, Speybroeck N. (2014) The burden of parasitic zoonoses in Nepal: A systematic review. PLoS Neglected Tropical Diseases; 8(1): e2634.

Díaz JM, Fernández G, Prieto A, Valverde S, Lago N, Díaz P, Panadero R, López C, Morrondo P, Díez-Baños P. (2014) Epidemiology of reproductive pathogens in semi-intensive lamb-

producing flocks in North-West Spain: A comparative serological study. Veterinary Journal; 200(2): 335-338.

Djokic V, Klun I, Musella V, Rinaldi L, Cringoli G, Sotiraki S, Djurkovic-Djakovic O. (2014) Spatial epidemiology of *Toxoplasma gondii* infection in goats in Serbia. Geospatial Health; 8(2): 479-488.

Do Amaral RP, Do Amaral RP, De Saidneuy AEKT, Ribeiro WL, De Andrade J. (2008) Serological profile of potential solid organ donors in Santa Catarina, Brazil. Transplantation Proceedings; 40(3): 665-667.

Dogan K, Kafkasli A, Karaman U, Atambay M, Karaoglu L, Colak C. (2012) The rates of seropositivity and seroconversion of toxoplasma infection in pregnant women. Mikrobiyoloji Bulteni; 46(2): 290-294.

Domingos A, Ito LS, Coelho E, Lucio JM, Matida LH, Ramos AN, Jr. (2013) Seroprevalence of *Toxoplasma gondii* IgG antibody in HIV/AIDS-infected individuals in Maputo, Mozambique. Revista De Saude Publica; 47(5): 890-896.

Dubey JP, Beattie CP. (1988) Toxoplasmosis of Animals and Man. Boca Raton, Florida: CRC Press.

Dubey JP, Kotula AW, Sharar A, Andrews CD, Lindsay DS. (1990) Effect of high temperature on infectivity of *Toxoplasma gondii* tissue cysts in pork. Journal of Parasitology; 76(2): 201-204.

Dubey JP. (1994) Toxoplasmosis. Journal of the American Veterinary Medical Association; 205(11): 1593-1598.

Dubey JP. (1995) Duration of immunity to shedding of *Toxoplasma gondii* oocysts by cats. Journal of Parasitology; 81(3): 410-415.

Dubey JP. (1996) *Toxoplasma gondii.* Section IV, Parasitology. In: S Baron (eds). Medical Microbiology. 4th edition. UTMB.

Dubey JP. (2001) Oocyst shedding by cats fed isolated bradyzoites and comparison of infectivity of bradyzoites of the VEG strain *Toxoplasma gondii* to cats and mice. Journal of Parasitology; 87(1): 215-219.

Dubey JP, Graham DH, Blackston CR, Lehmann T, Gennari SM, Ragozo AMA, Nishi SM, Shen SK, Kwok OCH, Hill DE, Thulliez P. (2002) Biological and genetic characterisation of *Toxoplasma gondii* isolates from chickens (*Gallus domesticus*) from São Paulo, Brazil: Unexpected findings. International Journal for Parasitology; 32(1): 99-105.

Dubey JP, Sundar N, Hill D, Velmurugan GV, Bandini LA, Kwok OCH, Majumdar D, Su C. (2008) High prevalence and abundant atypical genotypes of *Toxoplasma gondii* isolated from lambs destined for human consumption in the USA. International Journal for Parasitology; 38(8–9): 999-1006.

Dubey JP, Su C. (2009) Population biology of *Toxoplasma gondii:* What's out and where did they come from. Memorias do Instituto Oswaldo Cruz; 104(2): 190-195.

Dubey JP. (2010) *Toxoplasma gondii* infections in chickens (*Gallus domesticus*): prevalence, clinical disease, diagnosis and public health significance. Zoonoses and Public Health; 57: 60-73.

Dubey JP, Rajendran C, Ferreira LR, Martins J, Kwok OCH, Hill DE, Villena I, Zhou H, Su C, Jones JL. (2011) High prevalence and genotypes of *Toxoplasma gondii* isolated from goats, from a retail meat store, destined for human consumption in the USA. International Journal for Parasitology; 41(8): 827-833.

Dubey JP, Hill DE, Rozeboom DW, Rajendran C, Choudhary S, Ferreira LR, Kwok OCH, Su C. (2012a) High prevalence and genotypes of *Toxoplasma gondii* isolated from organic pigs in northern USA. Veterinary Parasitology; 188(1–2): 14-18.

Dubey JP, Lago EG, Gennari SM, Su C, Jones JL. (2012b) Toxoplasmosis in humans and animals in Brazil: high prevalence, high burden of disease, and epidemiology. Parasitology; 139(11): 1375-1424.

Dubey JP, Casey SJ, Zajac AM, Wildeus SA, Lindsay DS, Verma SK, Oliveira S, Kwok OCH, Su C. (2014a) Isolation and genetic characterization of *Toxoplasma gondii* from alpaca (*Vicugna pacos*) and sheep (*Ovis aries*). Tropical Animal Health and Production; 46(8): 1503-1507.

Dubey JP, Hotea I, Olariu TR, Jones JL, Dărăbuş G. (2014b) Epidemiological review of toxoplasmosis in humans and animals in Romania. Parasitology; 141(03): 311-325.

EFSA. (2007) Surveillance and monitoring of Toxoplasma in humans, food and animals. Scientific Opinion of the Panel on Biological Hazards. EFSA Journal; 583: 1-64.

EFSA. (2011) Scientific Opinion on the public health hazards to be covered by inspection of meat (swine). EFSA Panel on Biological Hazards (BIOHAZ), EFSA Panel on Contaminants in the Food Chain (CONTAM), EFSA Panel on Animal Health and Welfare (AHAW). EFSA Journal; 9(10): 2351.

EFSA. (2013a) Scientific Opinion on the public health hazards to be covered by inspection of meat from sheep and goats. EFSA Panel on Biological Hazards (BIOHAZ), with the contribution of the Panels on Contaminants in the Food Chain (CONTAM) and Animal Health and Welfare (AHAW). EFSA Journal; 11(6): 3265.

EFSA. (2013b) Scientific Opinion on the public health hazards to be covered by inspection of meat (bovine animals). EFSA Panel on Biological Hazards (BIOHAZ) with the contribution of the EFSA Panels on Contaminants in the Food Chain (CONTAM) and Animal Health and Welfare (AHAW). EFSA Journal; 11(6): 3266.

EFSA. (2013c) Scientific Opinion on the public health hazards to be covered by inspection of meat from farmed game. EFSA Panel on Biological Hazards (BIOHAZ) with the contribution of the EFSA Panels on Contaminants in the Food Chain (CONTAM) and Animal Health and Welfare (AHAW). EFSA Journal; 11(6): 3264.

Eisa ZM, Agag A, Al-Dawsary MM, Eifan SA, Al-Sum BA. (2013) Toxoplasmosis in women, men, infants and animals in Jazan District. Biosciences Biotechnology Research Asia; 10(1): 205-211.

El-Nawawi FA, Tawfik MA, Shaapan RM. (2008) Methods for inactivation of *Toxoplasma gondii* cysts in meat and tissues of experimentally infected sheep. Foodborne Pathogens and Disease; 5(5): 687-690.

Elbez-Rubinstein A, Ajzenberg D, Dardé M-L, Cohen R, Dumètre A, Yera H, Gondon E, Janaud J-C, Thulliez P. (2009) Congenital toxoplasmosis and reinfection during pregnancy: Case report, strain characterization, experimental model of reinfection, and review. Journal of Infectious Diseases; 199(2): 280-285.

Elfahal AM, Elhassan AM, Hussien MO, Enan KA, Musa AB, El Hussein AM. (2013) Seroprevalence of *Toxoplasma gondii* in dairy cattle with reproductive problems in Sudan. ISRN Veterinary Science; 2013: 895165.

Elsheikha HM, Azab MS, Abousamra NK, Rahbar MH, Elghannam DM, Raafat D. (2009) Seroprevalence of and risk factors for *Toxoplasma gondii* antibodies among asymptomatic blood donors in Egypt. Parasitology Research; 104(6): 1471-1476.

Enrique Gomez-Marin J, de-la-Torre A, Barrios P, Cardona N, Alvarez C, Herrera C. (2012) Toxoplasmosis in military personnel involved in jungle operations. Acta Tropica; 122(1): 46-51.

Ergin S, Ciftcioglu G, Midilli K, Issa G, Gargili A. (2009) Detection of *Toxoplasma gondii* from meat and meat products by the nested-PCR method and its relationship with seroprevalence in slaughtered animals. Bulletin of the Veterinary Institute in Pulawy; 53(4): 657-661.

Fajardo HV, D'Avila S, Bastos RR, Cyrino CD, Detoni MdL, Garcia JL, das Neves LB, Nicolau JL, Reis Amendoeira MR. (2013) Seroprevalence and risk factors of toxoplasmosis in cattle from extensive and semi-intensive rearing systems at Zona da Mata, Minas Gerais state, Southern Brazil. Parasites and Vectors; 6: 191.

Fallah E, Hajizadeh M, Farajnia S, Khanmahammadi M. (2011) SAG2 locus genotyping of *Toxoplasma gondii* in meat products of East Azerbaijan Province, North West of Iran during 2010-2011. African Journal of Biotechnology; 10(62): 13631-13635.

Feitosa TF, Vilela VLR, Bezerra de Melo LR, de Almeida Neto JL, de Oliveira Souto DV, de Morais DF, Athayde ACR, Azevedo SS, de Jesus Pena HF. (2014) *Toxoplasma gondii* and *Neospora caninum* in slaughtered pigs from Northeast, Brazil. Veterinary Parasitology; 202(3–4): 305-309.

Ferguson W, Mayne PD, Cafferkey M, Butler K. (2011) Lack of awareness of risk factors for primary toxoplasmosis in pregnancy. Irish Journal of Medical Science; 180(4): 807-811.

Ferreira-da-Silva MdF, Takács AC, Barbosa HS, Gross U, Lüder CGK. (2009) Primary skeletal muscle cells trigger spontaneous *Toxoplasma gondii* tachyzoite-to-bradyzoite conversion at higher rates than fibroblasts. International Journal of Medical Microbiology; 299(5): 381-388.

Ferreira AIC, De Mattos CCB, Frederico FB, Meira CS, Almeida GCJ, Nakashima F, Bernardo CR, Pereira-Chioccola VL, De Mattos LC. (2014) Risk factors for ocular toxoplasmosis in Brazil. Epidemiology and Infection; 142(01): 142-148.

Ferreira MU, Hiramoto RM, Aureliano DP, da Silva-Nunes M, da Silva NS, Malafronte RS, Muniz PT. (2009) A community-based survey of human toxoplasmosis in rural Amazonia: Seroprevalence, seroconversion rate, and associated risk factors. American Journal of Tropical Medicine and Hygiene; 81(1): 171-176.

Flatt A, Shetty N. (2013) 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; 23(4): 648-652.

Forsyth MB, Morris AJ, Sinclair DA, Pritchard CP. (2012) Investigation of zoonotic infections among Auckland Zoo staff: 1991–2010. Zoonoses and Public Health; 59(8): 561-567.

Frazão-Teixeira E, de Oliveira FCR. (2011) Anti–*Toxoplasma gondii* antibodies in cattle and pigs in a highly endemic area for human toxoplasmosis in Brazil. Journal of Parasitology; 97(1): 44-47.

Frenkel JK. (1990) Transmission of toxoplasmosis and the role of immunity in limiting transmission and illness. Journal of the American Veterinary Medical Association; 196(2): 233-240.

Fuh YB, Lin CS, Liao AT, Pong YM, Tung MC, Fei CY, Lin DS. (2013) Survey of *Toxoplasma gondii* in Taipei: Livestock meats, internal organs, cat and dog sera. Thai Journal of Veterinary Medicine; 43(1): 15-21.

Fusco G, Rinaldi L, Guarino A, Proroga YTR, Pesce A, Giuseppina DM, Cringoli G. (2007) *Toxoplasma gondii* in sheep from the Campania region (Italy). Veterinary Parasitology; 149(3–4): 271-274.

Galván-Ramírez MDLL, Sánchez-Orozco LV, Rodríguez LR, Rodríguez S, Roig-Melo E, Troyo Sanromán R, Chiquete E, Armendáriz-Borunda J. (2013) Seroepidemiology of *Toxoplasma gondii* infection in drivers involved in road traffic accidents in the metropolitan area of Guadalajara, Jalisco, Mexico. Parasites and Vectors; 6(1): 294.

Galván-Ramirez ML, Madriz Elisondo AL, Rico Torres CP, Luna-Pastén H, Rodríguez Pérez LR, Rincón-Sánchez AR, Franco R, Salazar-Montes A, Correa D. (2010) Frequency of *Toxoplasma gondii* in pork meat in Ocotlán, Jalisco, Mexico. Journal of Food Protection; 73(6): 1121-1123.

García-Bocanegra I, Dubey JP, Simon-Grifé M, Cabezón O, Casal J, Allepuz A, Napp S, Almería S. (2010a) Seroprevalence and risk factors associated with *Toxoplasma gondii* infection in pig farms from Catalonia, north-eastern Spain. Research in Veterinary Science; 89(1): 85-87.

García-Bocanegra I, Simon-Grifé M, Dubey JP, Casal J, Martín GE, Cabezón O, Perea A, Almería S. (2010b) Seroprevalence and risk factors associated with *Toxoplasma gondii* in domestic pigs from Spain. Parasitology International; 59(3): 421-426.

García-Bocanegra I, Simon-Grifé M, Sibila M, Dubey JP, Cabezón O, Martín G, Almería S. (2010c) Duration of maternally derived antibodies in *Toxoplasma gondii* naturally infected piglets. Veterinary Parasitology; 170(1–2): 134-136.

García-Bocanegra I, Cabezón O, Hernández E, Martínez-Cruz MS, Martínez-Moreno Á, Martínez-Moreno J. (2013) *Toxoplasma gondii* in ruminant species (cattle, sheep, and goats) from southern Spain. Journal of Parasitology; 99(3): 438-440.

Garcia JL, Marques FAC, Vidotto O, Navarro IT, Martins GF, Zulpo DL, da Cunha IAL, Taroda A, Cardim ST, Ewald MPD. (2012) Sero-occurrence of anti-*Toxoplasma gondii* antibodies and vertical transmission in slaughtered beef cows (*Bos indicus*). Semina-Ciencias Agrarias; 33(3): 1095-1101.

Ge W, Sun HC, Wang ZD, Xu P, Wang W, Mu GD, Wei F, Liu Q. (2014) Prevalence and genotype of *Toxoplasma gondii* infection in cattle from Jilin Province, Northeastern China. Vector-Borne and Zoonotic Diseases; 14(6): 399-402.

Gebremedhin EZ, Agonafir A, Tessema TS, Tilahun G, Medhin G, Vitale M, Marco VD, Cox E, Vercruysse J, Dorny P. (2013) Seroepidemiological study of ovine toxoplasmosis in East and West Shewa Zones of Oromia Regional State, Central Ethiopia. BMC Veterinary Research; 9: 117.

Gebremedhin EZ, Abdurahaman M, Hadush T, Tessema TS. (2014a) Seroprevalence and risk factors of *Toxoplasma gondii* infection in sheep and goats slaughtered for human consumption in Central Ethiopia. BMC Research Notes; 7: 696.

Gebremedhin EZ, Abdurahaman M, Tessema TS, Tilahun G, Cox E, Goddeeris B, Dorny P, De Craeye S, Dardé ML, Ajzenberg D. (2014b) Isolation and genotyping of viable *Toxoplasma gondii* from sheep and goats in Ethiopia destined for human consumption. Parasites and Vectors; 7(1): 425.

Gencay YE, Yildiz K, Gokpinar S, Leblebicier A. (2013) A potential infection source for humans: Frozen buffalo meat can harbour tissue cysts of *Toxoplasma gondii*. Food Control; 30(1): 86-89.

George BP, Schneider EB, Venkatesan A. (2014) Encephalitis hospitalization rates and inpatient mortality in the United States, 2000-2010. PLoS ONE; 9(9): e104169.

Georgiadis MP, Johnson WO, Gardner IA, Singh R. (2003) Correlation-adjusted estimation of sensitivity and specificity of two diagnostic tests. Journal of the Royal Statistical Society Series C-Applied Statistics; 52: 63-76.

Ghoneim NH, Shalaby SI, Hassanain NA, Zeedan GSG, Soliman YA, Abdalhamed AM. (2009) Comparative study between serological and molecular methods for diagnosis of

toxoplasmosis in women and small ruminants in Egypt. Foodborne Pathogens and Disease; 7(1): 17-22.

Giadinis ND, Lafi SQ, Ioannidou E, Papadopoulos E, Terpsidis K, Karanikolas G, Petridou EJ, Brozos C, Karatzias H. (2013) Reduction of the abortion rate due to Toxoplasma in 3 goat herds following administration of sulfadimidine. Canadian Veterinary Journal; 54(11): 1080-1082.

Giangaspero M, Bonfini B, Orusa R, Savini G, Osawa T, Harasawa R, ocirc. (2013) Epidemiological Survey for *Toxoplasma gondii*, *Chlamydia psittaci* var. *ovis*, *Mycobacterium paratuberculosis*, *Coxiella burnetii*, *Brucella* spp., Leptospirosis and Orf Virus among sheep from Northern Districts of Japan. Journal of Veterinary Medical Science; 75(5): 679-684.

Gilbert R, Tan HK, Cliffe S, Guy E, Stanford M. (2006) Symptomatic toxoplasma infection due to congenital and postnatally acquired infection. Archives of Disease in Childhood; 91(6): 495-498.

Gilbert S, McIntyre L, Cressey P, Thomson B, Wilson M. (2010) Food safety during pregnancy. Evidence-based source document. ESR Client Report FW09089. Christchurch: ESR.

Gilot-Fromont E, Aubert D, Belkilani S, Hermitte P, Gibout O, Geers R, Villena I. (2009a) Landscape, herd management and within-herd seroprevalence of *Toxoplasma gondii* in beef cattle herds from Champagne-Ardenne, France. Veterinary Parasitology; 161(1–2): 36-40.

Gilot-Fromont E, Riche B, Rabilloud M. (2009b) Toxoplasma seroprevalence in a rural population in France: detection of a household effect. BMC Infectious Diseases; 9: 76.

Ginsbourger M, Guinard A, Villena I, King LA, El-Eid N, Schwoebel V. (2012) Collective outbreak of food poisoning due to *Toxoplasma gondii* associated with the consumption of lamb meat, Aveyron (France), November 2010. Bulletin Epidemiologique Hebdomadaire; 2012(16-17): 195-197.

Gkogka E, Reij MW, Havelaar AH, Zwietering MH, Gorris LGM. (2011) Risk-based estimate of effect of foodborne diseases on public health, Greece. Emerging Infectious Diseases; 17(9): 1581-1590.

Goldsmid J, Speare R, Bettiol S. (2003) The parasitology of food. In: AD Hocking (eds). Foodborne Microorganisms of Public Health Significance. 6th edition. Sydney: Australian Institute of Food Science and Technology Inc., NSW Branch, Food Microbiology Group.

Gomez-Samblas M, Vílchez S, Racero JC, Fuentes MV, Osuna A. (2015) Quantification and viability assays of *Toxoplasma gondii* in commercial "Serrano" ham samples using magnetic capture real-time qPCR and bioassay techniques. Food Microbiology; 46(0): 107-113.

Górecki MT, Andrzejewska I, Steppa R. (2008) Is order of voluntarily entrance to milking parlour related to *Toxoplasma gondii* infection in sheep - A brief note. Applied Animal Behaviour Science; 110(3–4): 392-396.

Gras L, Gilbert RE, Ades AE, Dunn DT. (2001) Effect of prenatal treatment on the risk of intracranial and ocular lesions in children with congenital toxoplasmosis. International Journal of Epidemiology; 30(6): 1309-1313.

Guerra NR, Alves B, Farias MPO, Mota RA, Alves LC. (2014) Frequency of *Toxoplasma gondii* antibodies in bovines in the state of Pernambuco, Brazil. Revista Brasileira De Parasitologia Veterinaria; 23(3): 417-419.

Gutierrez J, O'Donovan J, Williams E, Proctor A, Brady C, Marques PX, Worrall S, Nally JE, McElroy M, Bassett H, Sammin D, Buxton D, Maley S, Markey BK. (2010) Detection and quantification of *Toxoplasma gondii* in ovine maternal and foetal tissues from experimentally infected pregnant ewes using real-time PCR. Veterinary Parasitology; 172(1–2): 8-15.

Halos L, Thébault A, Aubert D, Thomas M, Perret C, Geers R, Alliot A, Escotte-Binet S, Ajzenberg D, Dardé M-L, Durand B, Boireau P, Villena I. (2010) An innovative survey underlining the significant level of contamination by *Toxoplasma gondii* of ovine meat consumed in France. International Journal for Parasitology; 40(2): 193-200.

Halová D, Mulcahy G, Rafter P, Turčeková L, Grant T, de Waal T. (2013) *Toxoplasma gondii* in Ireland: Seroprevalence and novel molecular detection method in sheep, pigs, deer and chickens. Zoonoses and Public Health; 60(2): 168-173.

Halsby K, Guy E, Said B, Francis J, O'Connor C, Kirkbride H, Morgan D. (2014) Enhanced surveillance for toxoplasmosis in England and Wales, 2008–2012. Epidemiology and Infection; 142(08): 1653-1660.

Hamidinejat H, Ghorbanpour M, Nabavi L, Hajikolaie MRH, Jalali MHR. (2010) Occurrence of anti-*Toxoplasma gondii* antibodies in female cattle in south-west of Iran. Tropical Animal Health and Production; 42(5): 899-903.

Hamilton CM, Katzer F, Innes EA, Kelly PJ. (2014) Seroprevalence of *Toxoplasma gondii* in small ruminants from four Caribbean islands. Parasites and Vectors; 7(1): 449.

Hassanain MA, Elfadaly HA, Shaapan RM, Hassanain NA, Barakat AM. (2011) Biological assay of *Toxoplasma gondii* Egyptian mutton isolates. International Journal of Zoological Research; 7(4): 330-337.

Havelaar AH, Haagsma JA, Mangen MJ, Kemmeren JM, Verhoef LP, Vijgen SM, Wilson M, Friesema IH, Kortbeek LM, van Duynhoven YT, van Pelt W. (2012) Disease burden of foodborne pathogens in the Netherlands, 2009. International Journal of Food Microbiology; 156(3): 231-238.

Hecker YP, Moore DP, Manazza JA, Unzaga JM, Spath EJA, Pardini LL, Venturini MC, Roberi JL, Campero CM. (2013) First report of seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in dairy sheep from Humid Pampa, Argentina. Tropical Animal Health and Production; 45(7): 1645-1647.

Hedman K, Lappalainen M, Seppäiä I, Mäkelä O. (1989) Recent primary toxoplasma infection indicated by a low avidity of specific IgG. Journal of Infectious Diseases; 159(4): 736-740.

Hernández-Cortazar IB, Jiménez-Coello M, Acosta-Viana KY, Guzmán-Marín E, Torres-Acosta JFJ, Rodríguez-Buenfil JC, Ortega-Pacheco A. (2014) Comparing the dynamics of *Toxoplasma gondii* seroconversion in growing sheep kept on raised slatted floor cages or floor pens in Yucatan, Mexico. Small Ruminant Research; 121(2–3): 400-403.

Heymann DL. (2004) Control of Communicable Diseases Manual. 18th edition. American Public Health Association.

Hide G, Morley EK, Hughes JM, Gerwash O, Elmahaishi MS, Elmahaishi KH, Thomasson D, Wright EA, Williams RH, Murphy RG, Smith JE. (2009) Evidence for high levels of vertical transmission in *Toxoplasma gondii*. Parasitology; 136(Special Issue 14): 1877-1885.

Hill D, Coss C, Dubey JP, Wroblewski K, Sautter M, Hosten T, Munoz-Zanzi C, Mui E, Withers S, Boyer K, Hermes G, Coyne J, Jagdis F, Burnett A, McLeod P, Morton H, Robinson D, McLeod R. (2011) Identification of a sporozoite-specific antigen from *Toxoplasma gondii*. Journal of Parasitology; 97(2): 328-337.

Hill DE, Dubey JP. (2003) *Toxoplasma gondii*. In: ME Torrence; RE Isaacson (eds). Microbial Food Safety in Animal Agriculture - Current Topics. Ames, Iowa: Iowa State Press.

Hill DE, Chirukandoth S, Dubey JP, Lunney JK, Gamble HR. (2006) Comparison of detection methods for *Toxoplasma gondii* in naturally and experimentally infected swine. Veterinary Parasitology; 141(1–2): 9-17.

Hill DE, Haley C, Wagner B, Gamble HR, Dubey JP. (2010) Seroprevalence of and risk factors for *Toxoplasma gondii* in the US swine herd using sera collected during the National Animal Health Monitoring Survey (Swine 2006). Zoonoses and Public Health; 57(1): 53-59.

Hofhuis A, Van Pelt W, Van Duynhoven YTHP, Nijhuis CDM, Mollema L, Van Der Klis FRM, Havelaar AH, Kortbeek LM. (2011) Decreased prevalence and age-specific risk factors for *Toxoplasma gondii* IgG antibodies in The Netherlands between 1995/1996 and 2006/2007. Epidemiology and Infection; 139(04): 530-538.

Holec-Gasior L, Kur J, Hiszczynska-Sawicka E, Drapala D, Dominiak-Gorski B, Pejsak Z. (2010) Application of recombinant antigens in serodiagnosis of swine toxoplasmosis and prevalence of *Toxoplasma gondii* infection among pigs in Poland. Polish Journal of Veterinary Sciences; 13(3): 457-464.

Holec-Gasior L, Drapala D, Dominiak-Gorski B, Kur J. (2013) Epidemiological study of *Toxoplasma gondii* infection among cattle in Northern Poland. Annals of Agricultural and Environmental Medicine; 20(4): 653-656.

Holliman RE, Raymond R, Renton N, Johnson JD. (1994) The diagnosis of toxoplasmosis using IgG avidity. Epidemiology & Infection; 112(02): 399-408.

Howe DK, Sibley LD. (1995) *Toxoplasma gondii* comprises three clonal lineages: Correlation of parasite genotype with human disease. Journal of Infectious Diseases; 172(6): 1561-1566.

Huang CQ, Lin YY, Dai AL, Li XH, Yang XY, Yuan ZG, Zhu XQ. (2010) Seroprevalence of *Toxoplasma gondii* infection in breeding sows in Western Fujian Province, China. Tropical Animal Health and Production; 42(1): 115-118.

Huong LT, Dubey JP. (2007) Seroprevalence of *Toxoplasma gondii* in pigs from Vietnam. Journal of Parasitology; 93(4): 951-952.

Hutchinson JP, Wear AR, Lambton SL, Smith RP, Pritchard GC. (2011) Survey to determine the seroprevalence of *Toxoplasma gondii* infection in British sheep flocks. Veterinary Record; 169(22): 582.

Innes EA, Bartley PM, Maley S, Katzer F, Buxton D. (2009) Veterinary vaccines against *Toxoplasma gondii*. Memorias do Instituto Oswaldo Cruz; 104(2): 246-251.

Inpankaew T, Pinyopanuwut N, Chimnoi W, Kengradomkit C, Sununta C, Zhang G, Nishikawa Y, Igarashi I, Xuan X, Jittapalapong S. (2010) Serodiagnosis of *Toxoplasma gondii* infection in dairy cows in Thailand. Transboundary and Emerging Diseases; 57(1-2): 42-45.

Iovu A, Györke A, Mircean V, Gavrea R, Cozma V. (2012) Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in dairy goats from Romania. Veterinary Parasitology; 186(3–4): 470-474.

Jaguaribe Ekman CC, do Valle Chiossi MF, Meireles LR, de Andrade Junior HF, Figueiredo WM, Moraes Marciano MA, de Albuquerque Luna EJ. (2012) Case-control study of an outbreak of acute toxoplasmosis in an industrial plant in the state of Sao Paulo, Brazil. Revista do Instituto de Medicina Tropical de Sao Paulo; 54(5): 239-244.

James BO, Agbonile IO, Okolo M, Lawani AO, Omoaregba JO. (2013) Prevalence of *Toxoplasma gondii* infection among individuals with severe mental illness in Nigeria: a case control study. Pathogens and Global Health; 107(4): 189-193.

Jayamaha JC, Robertson P, Rawlinson WD. (2012) Congenital toxoplasmosis over 10 years in a low-incidence population. Med J Aust; 196(7): 443-444.

Jiang H-H, Zhang W-B, Zhao L, Zhou D-H, Song H-Q, Xu C-M, Deng S-Z, Zhu X-Q. (2014) Seroprevalence of *Toxoplasma gondii* infection in pigs in Jiangxi Province, Southeastern China. Foodborne Pathogens and Disease; 11(5): 362-365.

Jimenez-Coello M, Guzman-Marin E, Ortega-Pacheco A, Acosta-Viana KY. (2011) Immunological status against *Toxoplasma gondii* in non-cat owners from an endemic region of Mexico. Vector-Borne and Zoonotic Diseases; 11(8): 1057-1061.

Jokelainen P, Näreaho A, Knaapi S, Oksanen A, Rikula U, Sukura A. (2010) *Toxoplasma gondii* in wild cervids and sheep in Finland: North-south gradient in seroprevalence. Veterinary Parasitology; 171(3–4): 331-336.

Jones JL, Kruszon-Moran D, Sanders-Lewis K, Wilson M. (2007) *Toxoplasma gondii* infection in the United States, 1999–2004, decline from the prior decade. American Journal of Tropical Medicine and Hygiene; 77(3): 405-410.

Jones JL, Dargelas V, Roberts J, Press C, Remington JS, Montoya JG. (2009) Risk factors for *Toxoplasma gondii* infection in the United States. Clinical Infectious Diseases; 49(6): 878-884.

Jones JL, Dubey JP. (2010) Waterborne toxoplasmosis--recent developments. Exp Parasitol; 124(1): 10-25.

Jones JL, Holland GN. (2010) Annual burden of ocular toxoplasmosis in the United States. American Journal of Tropical Medicine and Hygiene; 82(3): 464-465.

Jones JL, Kruszon-Moran D, Rivera HN, Price C, Wilkins PP. (2014a) *Toxoplasma gondii* seroprevalence in the United States 2009-2010 and comparison with the past two decades. American Journal of Tropical Medicine and Hygiene; 90(6): 1135-1139.

Jones JL, Shvachko VA, Wilkins EE, Bergen R, Manos MM. (2014b) Rate of congenital toxoplasmosis in large integrated health care setting, California, USA, 1998-2012. Emerging Infectious Diseases; 20(9): 1573-1574.

Juanah LY, Jalaludin J, Osman M, Osman ZJ. (2013) Seroprevalence of *Toxoplasma gondii* among schizophrenics at Hospital Kajang. American Journal of Infectious Diseases; 9(1): 11-16.

Katzer F, Brülisauer F, Collantes-Fernández E, Bartley PM, Burrells A, Gunn G, Maley SW, Cousens C, Innes EA. (2011) Increased *Toxoplasma gondii* positivity relative to age in 125 Scottish sheep flocks; Evidence of frequent acquired infection. Veterinary Research; 42(1): 121.

Katzer F, Canton G, Burrells A, Palarea-Albaladejo J, Horton B, Bartley PM, Pang Y, Chianini F, Innes EA, Benavides J. (2014) Immunization of lambs with the S48 strain of *Toxoplasma gondii* reduces tissue cyst burden following oral challenge with a complete strain of the parasite. Veterinary Parasitology; 205(1–2): 46-56.

Keith DF, Alex DWA. (2010) Seroprevalence of *Toxoplasma gondii* infection in goats from two counties in Mississippi, USA. Xii International Congress of Parasitology ICOPA, Melbourne, Australia.

Khamesipour F, Doosti A, Iranpour Mobarakeh H, Komba VGE. (2014) *Toxoplasma gondii* in cattle, camels and sheep in Isfahan and Chaharmahal va Bakhtiary provinces, Iran. Jundishapur Journal of Microbiology; 7(6): e17460.

Kijlstra A, Meerburg B, Cornelissen J, De Craeye S, Vereijken P, Jongert E. (2008) The role of rodents and shrews in the transmission of *Toxoplasma gondii* to pigs. Veterinary Parasitology; 156(3–4): 183-190.

Kirk M, Ford L, Glass K, Hall G. (2014) Foodborne illness, Australia, circa 2000 and circa 2010. Emerging Infectious Diseases; 20(11): 1857-1864.

Klun I, Vujanić M, Yera H, Nikolić A, Ivović V, Bobić B, Bradonjić S, Dupouy-Camet J, Djurković-Djaković O. (2011) *Toxoplasma gondii* infection in slaughter pigs in Serbia: seroprevalence and demonstration of parasites in blood. Veterinary Research; 42(1): 17-17.

Kortbeek LM, Hofhuis A, Nijhuis CDM, Havelaar AH. (2009) Congenital toxoplasmosis and DALYs in the Netherlands. Memorias do Instituto Oswaldo Cruz; 104(2): 370-373.

Kul O, Yildiz K, Ocal N, Freyre A, Deniz A, Karahan S, Atmaca HT, Gokpinar S, Dincel GC, Uzunalioğlu T, Terzi OS. (2013) In-vivo efficacy of toltrazuril on experimentally induced *Toxoplasma gondii* tissue cysts in lambs: A novel strategy for prevention of human exposure to meat-borne toxoplasmosis. Research in Veterinary Science; 94(2): 269-276.

Lago EG, Conrado GS, Piccoli CS, Carvalho RL, Bender AL. (2009) *Toxoplasma gondii* antibody profile in HIV-infected pregnant women and the risk of congenital toxoplasmosis. European Journal of Clinical Microbiology and Infectious Diseases; 28(4): 345-351.

Lake R, Gilbert S, Hudson A, Cressey P. (2008) Risk profile: *Toxoplasma gondii* in red meat and meat products. ESR Client Report FW06106. Christchurch: ESR.

Langoni H, Greca Júnior H, Guimarães FF, Ullmann LS, Gaio FC, Uehara RS, Rosa EP, Amorim RM, Da Silva RC. (2011) Serological profile of *Toxoplasma gondii* and *Neospora caninum* infection in commercial sheep from São Paulo State, Brazil. Veterinary Parasitology; 177(1–2): 50-54.

Lass A, Pietkiewicz H, Szostakowska B, Myjak P. (2009) Occurrence of *Toxoplasma gondii* parasite in food (meat, meat products, fruits and vegetables). Tropical Medicine and International Health; 14: 200-200.

Leal FE, Cavazzana CL, de Andrade HF, Jr., Galisteo AJ, Jr., De Mendonca JS, Kallas EG. (2007) *Toxoplasma gondii* pneumonia in immunocompetent subjects: case report and review. Clinical Infectious Diseases; 44(6): E62-E66.

Li H-L, Dong L, Li Q, Zhang L, Chen J, Zou F-C, Zhu X-Q. (2014) Seroepidemiology of *Toxoplasma gondii* infection in Bai and Han ethnic groups in southwestern China. Epidemiology and Infection; FirstView: 1-6.

Lin YL, Liao YS, Liao LR, Chen FN, Kuo HM, He SP. (2008) Seroprevalence and sources of *Toxoplasma* infection among indigenous and immigrant pregnant women in Taiwan. Parasitology Research; 103(1): 67-74.

Lindsay DS, Blagburn BL, Dubey JP. (2002) Survival of nonsporulated *Toxoplasma gondii* oocysts under refrigerator conditions. Veterinary Parasitology; 103(4): 309-313.

Liu Q, Wei F, Gao S, Jiang L, Lian H, Yuan B, Yuan Z, Xia Z, Liu B, Xu X, Zhu X-Q. (2009) *Toxoplasma gondii* infection in pregnant women in China. Transactions of The Royal Society of Tropical Medicine and Hygiene; 103(2): 162-166.

Liu X, Liu C, Liu Y, Jin H, Zhao Y, Chen J, Yang M, Liu Q. (2012) Seroprevalence of *Toxoplasma gondii* infection in slaughtered pigs and cattle in Liaoning Province, northeastern China. Journal of Parasitology; 98(2): 440-441.

Liz Stefen Sakata FB, Bellato V, Sartor AA, de Moura AB, de Souza AP, Farias JA. (2012) *Toxoplasma gondii* antibodies sheep in Lages, Santa Catarina, Brazil, and comparison using IFA and ELISA. Revista Brasileira De Parasitologia Veterinaria; 21(3): 196-200.

Lopes-Mori FMR, Mitsuka-Breganó R, Bittencourt LHFdB, Dias RCF, Gonçalves DD, Capobiango JD, Reiche EMV, Morimoto HK, Freire RL, Navarro IT. (2013) Gestational toxoplasmosis in Paraná State, Brazil: Prevalence of IgG antibodies and associated risk factors. Brazilian Journal of Infectious Diseases; 17(4): 405-409.

Lopes AP, Dubey JP, Moutinho O, Gargaté MJ, Vilares A, Rodrigues M, Cardoso L. (2012) Seroepidemiology of *Toxoplasma gondii* infection in women from the North of Portugal in their childbearing years. Epidemiology and Infection; 140(5): 872-877.

Lopes AP, Dubey JP, Neto F, Rodrigues A, Martins T, Rodrigues M, Cardoso L. (2013) Seroprevalence of *Toxoplasma gondii* infection in cattle, sheep, goats and pigs from the North of Portugal for human consumption. Veterinary Parasitology; 193(1–3): 266-269.

Lopes FMR, Goncalves DD, Dos Reis CR, Bregano RM, Freire RL, De Freitas JC, Navarro IT. (2008) Presence of domesticated cats and visual impairment associated to *Toxoplasma gondii* serum positive children at an elementary school in Jataizinho, state of Parana, Brazil. Revista Brasileira De Parasitologia Veterinaria; 17(1): 12-15.

Lopes WDZ, Santos TRd, da Silva RdS, Rossanese WM, de Souza FA, de Faria Rodrigues JDA, de Mendonça RP, Soares VE, Costa AJd. (2010) Seroprevalence of and risk factors for *Toxoplasma gondii* in sheep raised in the Jaboticabal microregion, São Paulo State, Brazil. Research in Veterinary Science; 88(1): 104-106.

Luciano DM, Menezes RC, Ferreira LC, Nicolau JL, das Neves LB, Luciano RM, Araji Dahroug MA, Reis Amendoeira MR. (2011a) Occurrence of anti-*Toxoplasma gondii* antibodies in cattle and pigs slaughtered, State of Rio de Janeiro. Revista Brasileira De Parasitologia Veterinaria; 20(4): 351-353.

Luciano DM, Menezes RC, Ferreira LC, Nicolau JL, das Neves LB, Luciano RM, Dahroug MAA, Amendoeira MRR. (2011b) Seroepidemiology of toxoplasmosis in goats and sheep from three counties of Rio de Janeiro state, Brazil. Pesquisa Veterinaria Brasileira; 31(7): 569-574.

Luft BJ, Remington JS. (1992) Toxoplasmic encephalitis in AIDS. Clinical Infectious Diseases; 15(2): 211-222.

Lundén A, Uggla A. (1992) Infectivity of *Toxoplasma gondii* in mutton following curing, smoking, freezing or microwave cooking. International Journal of Food Microbiology; 15(3-4): 357-363.

Maenz M, Schlueter D, Liesenfeld O, Schares G, Gross U, Pleyer U. (2014) Ocular toxoplasmosis past, present and new aspects of an old disease. Progress in Retinal and Eye Research; 39: 77-106.

Mainar-Jaime RC, Barberán M. (2007) Evaluation of the diagnostic accuracy of the modified agglutination test (MAT) and an indirect ELISA for the detection of serum antibodies against *Toxoplasma gondii* in sheep through Bayesian approaches. Veterinary Parasitology; 148(2): 122-129.

Mancianti F, Nardoni S, D'Ascenzi C, Pedonese F, Mugnaini L, Franco F, Papini R. (2013) Seroprevalence, detection of DNA in blood and milk, and genotyping of *Toxoplasma gondii* in a goat population in Italy. Biomed Research International; 2013: 905326.

Mangen M-JJ, Bouwknegt M, Friesema IHM, Haagsma JA, Kortbeek LM, Tariq L, Wilson M, van Pelt W, Havelaar AH. (2015) Cost-of-illness and disease burden of food-related pathogens in the Netherlands, 2011. International Journal of Food Microbiology; 196(0): 84-93.

Mason S, Quinnell RJ, Smith JE. (2010) Detection of *Toxoplasma gondii* in lambs via PCR screening and serological follow-up. Veterinary Parasitology; 169(3–4): 258-263.

Matsuo K, Kamai R, Uetsu H, Goto H, Takashima Y, Nagamune K. (2014) Seroprevalence of *Toxoplasma gondii* infection in cattle, horses, pigs and chickens in Japan. Parasitology International; 63(4): 638-639.

Meemken D, Tangemann AH, Meermeier D, Gundlach S, Mischok D, Greiner M, Klein G, Blaha T. (2014) Establishment of serological herd profiles for zoonoses and production diseases in pigs by "meat juice multi-serology". Preventive Veterinary Medicine; 113(4): 589-598.

Messerer L, Bouzbid S, Gourbdji E, Mansouri R, Bachi F. (2014) Séroprévalence de la toxoplasmose chez les femmes enceintes dans la wilaya d'Annaba, Algérie. Revue d'Épidémiologie et de Santé Publique; 62(2): 160-165.

Mie T, Pointon AM, Hamilton DR, Kiermeier A. (2008) A qualitative assessment of *Toxoplasma gondii* risk in ready-to-eat smallgoods processing. Journal of Food Protection; 71(7): 1442-1452.

Minbaeva G, Schweiger A, Bodosheva A, Kuttubaev O, Hehl AB, Tanner I, Ziadinov I, Torgerson PR, Deplazes P. (2013) *Toxoplasma gondii* infection in Kyrgyzstan: Seroprevalence, risk factor analysis, and estimate of congenital and AIDS-related toxoplasmosis. PLoS Neglected Tropical Diseases; 7(2): e2043.

Ministry for Primary Industries. (2014) IFRs & advisories. Accessed at: http://www.foodsafety.govt.nz/industry/importing/specific-foods/prescribed-foods/IFRs.htm. Accessed: 20 October 2014.

Ministry of Health. (2003) NZ Food NZ Children. Key results of the 2002 National Children's Nutrition Survey. Wellington: Ministry of Health.

Ministry of Health. (2008) Food and nutrition guidelines for healthy pregnant and breastfeeding women. A background paper. Wellington: Ministry of Health.

Mizgajska-Wiktor H, Jarosz W, Andrzejewska I, Krzykala M, Janowski J, Kozlowska M. (2013) Differences in some developmental features between *Toxoplasma gondii*-seropositive and seronegative school children. Folia Parasitologica; 60(5): 416-424.

Montoya JG, Liesenfeld O. (2004) Toxoplasmosis. Lancet; 363(9425): 1965-1976.

Morin L, Lobry JR, Peyron F, Wallon M. (2012) Seasonal variations in acute toxoplasmosis in pregnant women in the Rhône-Alpes region (France). Clinical Microbiology and Infection; 18(10): E401-E403.

Morris AJ, Croxson MC. (2004) Serological evidence of *Toxoplasma gondii* infection among pregnant women in Auckland. New Zealand Medical Journal; 117(1189): 770.

MPI. (2011) Scientific Evaluation of Bovine Post Mortem Examination Procedures in New Zealand Part B. Wellington New Zealand: Ministry for Primary Industries.

Munoz-Zanzi C, Tamayo R, Balboa J, Hill D. (2012) Detection of oocyst-associated toxoplasmosis in swine from southern Chile. Zoonoses and Public Health; 59(6): 389-392.

Munoz-Zanzi C, Williams-Nguyen J, Belongia EA. (2013) A sero-survey of toxoplasmosis in farm and non-farm children from Wisconsin, United States, 1997-1999. BMC Public Health; 13: 837.

Munoz-Zanzi CA, Fry P, Lesina B, Hill D. (2010) *Toxoplasma gondii* oocyst-specific antibodies and source of infection. Emerging Infectious Diseases; 16(10): 1591-1593.

Muraro LS, Caramori Junior JG, Amendoeira MR, Pereira JA, Filho JX, Vicente RT, Neves LB, Nicolau JL, Igarashi M, Moura ST. (2010) Seroprevalence of *Toxoplasma gondii* infection in swine matrices in Nova Mutum and Diamantino, Mato Grosso, Brazil. Revista Brasileira De Parasitologia Veterinaria; 19(4): 254-255.

Ndou RV, Maduna NM, Dzoma BM, Nyirenda M, Motsei LE, Bakunzi FR. (2013) A seroprevalance survey of *Toxoplasma gondii* amongst slaughter cattle in two high throughput abattoirs in the North West Province of South Africa. Journal of Food Agriculture and Environment; 11(1): 338-339.

Neumayerová H, Juránková J, Saláková A, Gallas L, Kovarčík K, Koudela B. (2014) Survival of experimentally induced *Toxoplasma gondii* tissue cysts in vacuum packed goat meat and dry fermented goat meat sausages. Food Microbiology; 39(0): 47-52.

New Zealand Pork. (2014) Pork Industry Review. Accessed at: http://www.nzpork.co.nz/images/custom/industry_review_september_2014.pdf. Accessed: 16 March 2014.

Nogareda F, Le Strat Y, Villena I, De Valk H, Goulet V. (2014) Incidence and prevalence of *Toxoplasma gondii* infection in women in France, 1980-2020: model-based estimation. Epidemiology and Infection; 142(8): 1661-1670.

Nowakowska D, Colón I, Remington JS, Grigg M, Golab E, Wilczynski J, Sibley LD. (2006) Genotyping of *Toxoplasma gondii* by multiplex PCR and peptide-based serological testing of samples from infants in Poland diagnosed with congenital toxoplasmosis. Journal of Clinical Microbiology; 44(4): 1382-1389.

Nunes FVA, Vaez JR, Pinheiro RR, Cavalcante ACR, Vitor RWA, Ahid SMM. (2013) Seroprevalence and factors associated with *Toxoplasma gondii* infection in goats on farms in

the municipality of Mossoro, Rio Grande do Norte, Brazil. Pesquisa Veterinaria Brasileira; 33(5): 565-570.

Ocal N, Babur C, Yagci BB, Macun HC, Celebi B, Kilic S, Yagci IP. (2008) Seroprevalance and co-existence of Brucellosis, Listeriosis and Toxoplasmosis in dairy cattle in Kirikkale province. Kafkas Universitesi Veteriner Fakultesi Dergisi; 14(1): 75-81.

Onyiche T, Ademola I. (2013) Seroprevalence of anti-*Toxoplasma gondii* antibodies in cattle and pigs in Ibadan, Nigeria. Journal of Parasitic Diseases: 1-6.

Opsteegh M, Langelaar M, Sprong H, den Hartog L, De Craeye S, Bokken G, Ajzenberg D, Kijlstra A, der Giessen Jv. (2010a) Direct detection and genotyping of *Toxoplasma gondii* in meat samples using magnetic capture and PCR. International Journal of Food Microbiology; 139(3): 193-201.

Opsteegh M, Teunis P, Mensink M, Züchner L, Titilincu A, Langelaar M, van der Giessen J. (2010b) Evaluation of ELISA test characteristics and estimation of *Toxoplasma gondii* seroprevalence in Dutch sheep using mixture models. Preventive Veterinary Medicine; 96(3–4): 232-240.

Opsteegh M, Prickaerts S, Frankena K, Evers EG. (2011a) A quantitative microbial risk assessment for meatborne Toxoplasma gondii infection in The Netherlands. International Journal of Food Microbiology; 150(2-3): 103-114.

Opsteegh M, Teunis P, Züchner L, Koets A, Langelaar M, van der Giessen J. (2011b) Low predictive value of seroprevalence of *Toxoplasma gondii* in cattle for detection of parasite DNA. International Journal for Parasitology; 41(3–4): 343-354.

Ortega-Pacheco A, Acosta Viana KY, Guzman-Marin E, Segura-Correa JC, Alvarez-Fleites M, Jimenez-Coello M. (2013) Prevalence and risk factors of *Toxoplasma gondii* in fattening pigs farm from Yucatan, Mexico. Biomed Research International; 2013: 231497.

Panadero R, Painceira A, López C, Vázquez L, Paz A, Díaz P, Dacal V, Cienfuegos S, Fernández G, Lago N, Díez-Baños P, Morrondo P. (2010) Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in wild and domestic ruminants sharing pastures in Galicia (Northwest Spain). Research in Veterinary Science; 88(1): 111-115.

Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents. (2014) Guidelines for the prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: Recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. Accessed at: http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf. Accessed: 10 March 2015.

Pappas G, Roussos N, Falagas ME. (2009) Toxoplasmosis snapshots: Global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. International Journal for Parasitology; 39(12): 1385-1394.

Paştiu A, Györke A, Blaga R, Mircean V, Rosenthal B, Cozma V. (2013) In Romania, exposure to *Toxoplasma gondii* occurs twice as often in swine raised for familial consumption as in

hunted wild boar, but occurs rarely, if ever, among fattening pigs raised in confinement. Parasitology Research; 112(6): 2403-2407.

Petersen E, Vesco G, Villari S, Buffalano W. (2010) What do we know about risk factors for infection in humans with *Toxoplasma gondii* and how can we prevent infections? Zoonoses and Public Health; 57: 8-17.

Pinheiro J, Jr., Mota R, da Fonseca Oliveira A, Faria E, Gondim L, da Silva A, Anderlini G. (2009) Prevalence and risk factors associated to infection by *Toxoplasma gondii* in ovine in the State of Alagoas, Brazil. Parasitology Research; 105(3): 709-715.

Poljak Z, Dewey CE, Friendship RM, Martin SW, Christensen J, Ojkic D, Wu J, Chow E. (2008) Pig and herd level prevalence of *Toxoplasma gondii* in Ontario finisher pigs in 2001, 2003, and 2004. Canadian Journal of Veterinary Research; 72(4): 303-310.

Pott S, Koethe M, Bangoura B, Zöller B, Daugschies A, Straubinger RK, Fehlhaber K, Ludewig M. (2013) Effects of pH, sodium chloride, and curing salt on the infectivity of *Toxoplasma gondii* tissue cysts. Journal of Food Protection; 76(6): 1056-1061.

Prangé A, Perret C, Marie JL, Calvet F, Halos L, Boireau P, Davoust B. (2009) [Toxoplasmosis: a survey on meat products in Cote d'Ivoire]. Medecine Tropicale; 69(6): 629-630.

Qiu JH, Wang CR, Zhang X, Sheng ZH, Chang QC, Zhao Q, Wu SM, Zou FC, Zhu XQ. (2012) Seroprevalence of *Toxoplasma gondii* in beef cattle and dairy cattle in Northeast China. Foodborne Pathogens and Disease; 9(7): 579-582.

Qu D, Zhou H, Han J, Tao S, Zheng B, Chi N, Su C, Du A. (2013) Development of reverse transcription loop-mediated isothermal amplification (RT-LAMP) as a diagnostic tool of *Toxoplasma gondii* in pork. Veterinary Parasitology; 192(1–3): 98-103.

Rahdar M, Samarbaf-Zadeh A, Arab L. (2012) Evaluating the prevalence of *Toxoplasma gondii* in meat and meat Products in Ahvaz by PCR method. Jundishapur Journal of Microbiology; 5(4): 570-573.

Rahman M, Azad MTA, Nahar L, Rouf SMA, Ohya K, Chiou S-P, Baba M, Kitoh K, Takashima Y. (2014) Age-specificity of *Toxoplasma gondii* seroprevalence in sheep, goats and cattle on subsistence farms in Bangladesh. Journal of Veterinary Medical Science; 76(9): 1257-1259.

Remington JS, McLeod R, Desmonts G. (1995) Toxoplasmosis. In: JS Remington; JO Klein (eds). Infectious Diseases of the Fetus and Newborn Infant. Philadelphia W.B. Saunders Co.

Robert-Gangneux F, Darde ML. (2012) Epidemiology of and diagnostic strategies for toxoplasmosis. Clin Microbiol Rev; 25(2): 264-296.

Roqueplo C, Halos L, Cabre O, Davoust B. (2011) *Toxoplasma gondii* in wild and domestic animals from New Caledonia. Parasite; 18(4): 345-348.

Russell DG, Parnell WR, Wilson NC, Faed J, Ferguson E, Herbison P, Horwath C, Nye T, Reid P, Walker R, Wilson B, Tukuitonga C. (1999) NZ Food: NZ People. Wellington: Ministry of Health.

Sagel U, Mikolajczyk RT, Kramer A. (2010) Seasonal trends in acute toxoplasmosis in pregnancy in the federal state of Upper Austria. Clinical Microbiology and Infection; 16(5): 516-517.

Sakikawa M, Noda S, Hanaoka M, Nakayama H, Hojo S, Kakinoki S, Nakata M, Yasuda T, Ikenoue T, Kojima T. (2012) Anti-*Toxoplasma* antibody prevalence, primary infection rate, and risk factors in a study of toxoplasmosis in 4,466 pregnant women in Japan. Clinical and Vaccine Immunology; 19(3): 365-367.

Samico Fernandes EF, Samico Fernandes MF, Kim PC, de Albuquerque PP, de Souza Neto OL, de SSA, de Moraes EP, de Morais EG, Mota RA. (2012) Prevalence of *Toxoplasma gondii* in slaughtered pigs in the state of Pernambuco, Brazil. Journal of Parasitology; 98(3): 690-691.

Samra NA, McCrindle CME, Penzhorn BL, Cenci-Goga B. (2007) Seroprevalence of toxoplasmosis in sheep in South Africa. Journal of the South African Veterinary Association; 78(3): 116-120.

Santos C, de Azevedo SS, Soares HS, Higino SSD, Pena HFD, Alves CJ, Gennari SM. (2012) Risk factors associated with *Toxoplasma gondii* seroprevalence in goats in the State of Paraiba, Brazil. Revista Brasileira De Parasitologia Veterinaria; 21(4): 399-404.

Santos TR, Costa AJ, Toniollo GH, Luvizotto MCR, Benetti AH, Santos RR, Matta DH, Lopes WDZ, Oliveira JA, Oliveira GP. (2009) Prevalence of anti-*Toxoplasma gondii* antibodies in dairy cattle, dogs, and humans from the Jauru micro-region, Mato Grosso state, Brazil. Veterinary Parasitology; 161(3–4): 324-326.

Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM. (2011) Foodborne illness acquired in the United States-major pathogens. Emerging Infectious Diseases; 17(1): 7-15.

Scallan E, Hoekstra RM, Mahon BE, Jones TF, Griffin PM. (2015) An assessment of the human health impact of seven leading foodborne pathogens in the United States using disability adjusted life years. Epidemiol Infect: 1-10.

Schoonman LB, Wilsmore T, Swai ES. (2010) Sero-epidemiological investigation of bovine toxoplasmosis in traditional and smallholder cattle production systems of Tanga Region, Tanzania. Tropical Animal Health and Production; 42(4): 579-587.

Schurer JM, Ndao M, Skinner S, Irvine J, Elmore SA, Epp T, Jenkins EJ. (2013) Parasitic Zoonoses: One Health Surveillance in Northern Saskatchewan. PLoS Neglected Tropical Diseases; 7(3): e2141.

Sechi P, Ciampelli A, Cambiotti V, Veronesi F, Cenci-Goga BT. (2013) Seroepidemiological study of toxoplasmosis in sheep in rural areas of the Grosseto district, Tuscany, Italy. Italian Journal of Animal Science; 12(2): 236-239.

Shaapan RM, El-Nawawi FA, Tawfik MAA. (2008) Sensitivity and specificity of various serological tests for the detection of *Toxoplasma gondii* infection in naturally infected sheep. Veterinary Parasitology; 153(3–4): 359-362.

Shahiduzzaman M, Islam MR, Khatun MM, Batanova TA, Kitoh K, Takashima Y. (2011) *Toxoplasma gondii s*eroprevalence in domestic animals and humans in Mymensingh District, Bangladesh. Journal of Veterinary Medical Science; 73(10): 1375-1376.

Sharif M, Daryani A, Barzegar G, Nasrolahei M. (2010) A seroepidemiological survey for toxoplasmosis among schoolchildren of Sari, Northern Iran. Tropical Biomedicine; 27(2): 220-225.

Sharma S, Sandhu KS, Bal MS, Kumar H, Verma S, Dubey JP. (2008) Serological survey of antibodies to *Toxoplasma gondii* in sheep, cattle, and buffaloes in Punjab, India. Journal of Parasitology; 94(5): 1174-1175.

Shobab L, Pleyer U, Johnsen J, Metzner S, James ER, Torun N, Fay MP, Liesenfeld O, Grigg ME. (2013) Toxoplasma serotype is associated with development of ocular toxoplasmosis. Journal of Infectious Diseases; 208(9): 1520-1528.

Silva RCd, Langoni H, Su C, Silva AVd. (2011) Genotypic characterization of *Toxoplasma gondii* in sheep from Brazilian slaughterhouses: New atypical genotypes and the clonal type II strain identified. Veterinary Parasitology; 175(1–2): 173-177.

Silveira Barbosa de Macedo MF, Barbosa de Macedo CA, de Barros LD, Martins GF, Sandeski LM, Zulpo DL, Leme da Cunha IA, Taroda A, Cardim ST, Garcia JL. (2012) Serum occurrence of anti-*Toxoplasma gondii* antibodies in dairy cows slaughtered in an abattoir for human consume. Ciencia Rural; 42(6): 1065-1069.

Sitoe SPBL, Rafael B, Meireles LR, de Andrade HF, Jr., Thompson R. (2010) Preliminary report of HIVand *Toxoplasma gondii* occurrence in pregnant women from Mozambique. Revista do Instituto de Medicina Tropical de Sao Paulo; 52(6): 291-295.

Smith JL. (1997) Long-term consequences of foodborne toxoplasmosis: Effects on the unborn, the immunocompromised, the elderly, and the immunocompetent. Journal of Food Protection; 60(12): 1595-1611.

Song E-S, Song K-H, Jung S-I, Park B-K, You M-J, Kim D-H. (2011) Latex agglutination test based prevalence of *Toxoplasma gondii* in native Korean cattle. Korean Journal of Veterinary Research; 51(1): 59-61.

Spagnol FH, Paranhos EB, Oliveira LLD, de Medeiros SM, Lopes CWG, Albuquerque GR. (2009) Prevalence of antibodies anti-*Toxoplasma gondii* in slaughtered cattle at stockyards in the State of Bahia, Brazil. Revista Brasileira De Parasitologia Veterinaria; 18(2): 42-45.

Sroka J, Karamon J, Cencek T, Dutkiewicz J. (2011) Preliminary assessment of usefulness of cELISA test for screening pig and cattle populations for presence of antibodies against *Toxoplasma gondii*. Annals of Agricultural and Environmental Medicine; 18(2): 335-339.

Statistics New Zealand. (2014) Agricultural Production Statistics: June 2014 (provisional). Accessed at: http://www.stats.govt.nz/browse_for_stats/industry_sectors/agriculture-horticulture-forestry/AgriculturalProduction_HOTPJun14prov.aspx. Accessed: 27 February 2015.

Steinparzer R, Reisp K, Grünberger B, Köfer J, Schmoll F, Sattler T. (2015) Comparison of different commercial serological tests for the detection of *Toxoplasma gondii* antibodies in serum of naturally exposed pigs. Zoonoses and Public Health; 62(2): 119-124.

Stimbirys A, Bagdonas J, Gerulis G, Russo P. (2007) A serological study on the prevalence of *Toxoplasma gondii* in sheep of Lithuania. Polish Journal of Veterinary Sciences; 10(2): 83-87.

Stormoen M, Tharaldsen J, Hopp P. (2012) Seroprevalence of *Toxoplasma gondii* infection in Norwegian dairy goats. Acta Veterinaria Scandinavica; 54: 3.

Su C, Zhang X, Dubey JP. (2006) Genotyping of *Toxoplasma gondii* by multilocus PCR-RFLP markers: A high resolution and simple method for identification of parasites. International Journal for Parasitology; 36(7): 841-848.

Swai ES, Kaaya JE. (2012) A survey of *Toxoplasma gondii* antibodies by latex agglutination assay in dairy goats in Northern Tanzania. Tropical Animal Health and Production; 45(1): 211-217.

Swierzy IJ, Muhammad M, Kroll J, Abelmann A, Tenter AM, Lüder CGK. (2014) *Toxoplasma gondii* within skeletal muscle cells: a critical interplay for food-borne parasite transmission. International Journal for Parasitology; 44(2): 91-98.

Taila AK, Hingwe AS, Johnson LE. (2011) Toxoplasmosis in a patient who was immunocompetent: A case report. Journal of Medical Case Reports; 5: 16.

Tao Q, Wang Z, Feng H, Fang R, Nie H, Hu M, Zhou Y, Zhao J. (2011) Seroprevalence and risk factors for *Toxoplasma gondii* infection on pig farms in central China. Journal of Parasitology; 97(2): 262-264.

Thomas MK, Murray R, Flockhart L, Pintar K, Pollari F, Fazil A, Nesbitt A, Marshall B. (2013) Estimates of the burden of foodborne illness in Canada for 30 specified pathogens and unspecified agents, Circa 2006. Foodborne Pathogens and Disease; 10(7): 639-648.

Torgerson PR, Mastroiacovo P. (2013) The global burden of congenital toxoplasmosis: A systematic review. Bulletin of the World Health Organization; 91(7): 501-508.

Torrey EF, Bartko JJ, Lun Z-R, Yolken RH. (2007) Antibodies to *Toxoplasma gondii* in patients with schizophrenia: A meta-analysis. Schizophrenia Bulletin; 33(3): 729-736.

Torrey EF, Bartko JJ, Yolken RH. (2012) *Toxoplasma gondii* and other risk factors for schizophrenia: An update. Schizophrenia Bulletin; 38(3): 642-647.

Tsai YJ, Chung WC, Fei AC, Kaphle K, Peng S, Wu YL. (2007) Seroprevalence of *Toxoplasma gondii* in pigs from slaughterhouses in Taiwan. Journal of Parasitology; 93(6): 1540-1541.

Turčeková Ľ, Antolová D, Reiterová K, Spišák F. (2013) Occurrence and genetic characterization of *Toxoplasma gondii* in naturally infected pigs. Acta Parasitologica; 58(3): 361-366.

Tzanidakis N, Maksimov P, Conraths FJ, Kiossis E, Brozos C, Sotiraki S, Schares G. (2012) *Toxoplasma gondii* in sheep and goats: Seroprevalence and potential risk factors under dairy husbandry practices. Veterinary Parasitology; 190(3–4): 340-348.

Ueno TEH, Gonçalves VSP, Heinemann MB, Dilli TLB, Akimoto BM, de Souza SLP, Gennari SM, Soares RM. (2009) Prevalence of *Toxoplasma gondii* and *Neospora caninum* infections in sheep from Federal District, central region of Brazil. Tropical Animal Health and Production; 41(4): 547-552.

University of Otago and Ministry of Health. (2011) A focus on nutrition: Key findings of the 2008/09 New Zealand Adult Nutrition Survey. Wellington: Ministry of Health.

Vally H, Glass K, Ford L, Hall G, Kirk MD, Shadbolt C, Veitch M, Fullerton KE, Musto J, Becker N. (2014) Proportion of illness acquired by foodborne transmission for nine enteric pathogens in Australia: An expert elicitation. Foodborne Pathogens and Disease; 11(9): 727-733.

van der Giessen J, Fonville M, Bouwknegt M, Langelaar M, Vollema A. (2007) Seroprevalence of *Trichinella spiralis* and *Toxoplasma gondii* in pigs from different housing systems in The Netherlands. Veterinary Parasitology; 148(3–4): 371-374.

Varela-Villalobos R, Rojas-Granados J, Valerio-Campos I, Chinchilla-Carmona M. (2013) Estado actual de la trasmisión de la toxoplasmosis por productos cárnicos en Costa Rica. Acta Médica Costarricense; 55(2): 82-86.

Varella IS, Canti ICT, Santos BR, Coppini AZ, Argondizzo LC, Tonin C, Wagner MB. (2009) Prevalence of acute toxoplasmosis infection among 41,112 pregnant women and the mother-to-child transmission rate in a public hospital in South Brazil. Memorias do Instituto Oswaldo Cruz; 104(2): 383-388.

Verhelst D, De Craeye S, Vanrobaeys M, Czaplicki G, Dorny P, Cox E. (2014) Seroprevalence of *Toxoplasma gondii* in domestic sheep in Belgium. Veterinary Parasitology; 205(1–2): 57-61.

Veronesi F, Ranucci D, Branciari R, Miraglia D, Mammoli R, Fioretti DP. (2011) Seroprevalence and risk factors for *Toxoplasma gondii i*nfection on finishing swine reared in the Umbria Region, Central Italy. Zoonoses and Public Health; 58(3): 178-184.

Vesco G, Buffolano W, La Chiusa S, Mancuso G, Caracappa S, Chianca A, Villari S, Currò V, Liga F, Petersen E. (2007) *Toxoplasma gondii* infections in sheep in Sicily, southern Italy. Veterinary Parasitology; 146(1–2): 3-8.

Villari S, Vesco G, Petersen E, Crispo A, Buffolano W. (2009) Risk factors for toxoplasmosis in pigs bred in Sicily, Southern Italy. Veterinary Parasitology; 161(1–2): 1-8.

Villena I, Durand B, Aubert D, Blaga R, Geers R, Thomas M, Perret C, Alliot A, Escotte-Binet S, Thébault A, Boireau P, Halos L. (2012) New strategy for the survey of *Toxoplasma gondii* in meat for human consumption. Veterinary Parasitology; 183(3–4): 203-208.

Vitale M, Tumino G, Partanna S, La Chiusa S, Mancuso G, Giglia ML, Presti VDML. (2014) Impact of traditional practices on food safety: A case of acute toxoplasmosis related to the consumption of contaminated raw pork sausage in Italy. Journal of Food Protection; 77(4): 643-646.

Walle F, Kebede N, Tsegaye A, Kassa T. (2013) Seroprevalence and risk factors for toxoplasmosis in HIV infected and non-infected individuals in Bahir Dar, Northwest Ethiopia. Parasites & Vectors; 6: 15.

Wallon M, Garweg JG, Abrahamowicz M, Cornu C, Vinault S, Quantin C, Bonithon-Kopp C, Picot S, Peyron F, Binquet C. (2014) Ophthalmic outcomes of congenital toxoplasmosis followed until adolescence. Pediatrics; 133(3): E601-E608.

Wang CR, Qiu JH, Gao JF, Liu LM, Wang C, Liu Q, Yan C, Zhu XQ. (2011) Seroprevalence of *Toxoplasma gondii* infection in sheep and goats in northeastern China. Small Ruminant Research; 97(1–3): 130-133.

Wang H, Wang T, Luo Q, Huo X, Wang L, Liu T, Xu X, Wang Y, Lu F, Lun Z, Yu L, Shen J. (2012) Prevalence and genotypes of *Toxoplasma gondii* in pork from retail meat stores in Eastern China. International Journal of Food Microbiology; 157(3): 393-397.

West DM. (2002) Ovine abortion in New Zealand. New Zealand Veterinary Journal; 50(3): 93-95.

Wiengcharoen J, Nakthong C, Mitchaothai J, Udonsom R, Sukthana Y. (2012) Toxoplasmosis and neosporosis among beef cattle slaughtered for food in western Thailand. Southeast Asian Journal of Tropical Medicine and Public Health; 43(5): 1087-1093.

Wong WK, Upton A, Thomas MG. (2013) Neuropsychiatric symptoms are common in immunocompetent adult patients with *Toxoplasma gondii* acute lymphadenitis. Scandinavian Journal of Infectious Diseases; 45(5): 357-361.

Wu D, Lv R, Sun X, Shu F, Zhou Z, Nie K, Duan G, Zou F. (2012) Seroprevalence of *Toxoplasma gondii* antibodies from slaughter pigs in Chongqing, China. Tropical Animal Health and Production; 44(4): 685-687.

Wu S-M, Danba C, Huang S-Y, Zhang D-L, Chen J, Gong G, Xu M-J, Yuan Z-G, Zhu X-Q. (2011) Seroprevalence of *Toxoplasma gondii* infection in Tibetan sheep in Tibet, China. Journal of Parasitology; 97(6): 1188-1189.

Xu MJ, Liu QY, Fu JH, Nisbet AJ, Shi DS, He XH, Pan Y, Zhou DH, Song HQ, Zhu XQ. (2012) Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* infection in dairy cows in subtropical southern China. Parasitology; 139(11): 1425-1428.

Xu Y, Li RC, Liu GH, Cong W, Zhang XX, Yu XL, Zhu XQ. (2014) Seroprevalence of *Toxoplasma gondii* infection in sows in Hunan province, China. Scientific World Journal; 2014: 347908.

Yaglom HD, Rottinghaus AA, Pithua P. (2014) Evidence of *Toxoplasma gondii e*xposure in Boer goat herds in Missouri, USA. Zoonoses and Public Health; 61(6): 395-397.

Yang N, Li H, He J, Mu M, Yang S. (2013) Seroprevalence of *Toxoplasma gondii* infection in domestic sheep in Liaoning Province, Northeastern China. Journal of Parasitology; 99(1): 174-175.

Yildiz K, Kul O, Gokpinar S, Atmaca HT, Gencay YE, Gazyagci AN, Babur C, Gurcan IS. (2014) The relationship between seropositivity and tissue cysts in sheep naturally infected with *Toxoplasma gondii*. Turkish Journal of Veterinary and Animal Sciences; 38(2): 169-175.

Youssefi MR, Sefidgar SAA, Ghaffari S. (2007) Seroepidemiology of sheep toxoplasmosis in Babol Northern Iran 2004. Pakistan Journal of Biological Sciences; 10(7): 1147-1148.

Yu J, Xia Z, Liu Q, Liu J, Ding J, Zhang W. (2007) Seroepidemiology of *Neospora caninum* and *Toxoplasma gondii* in cattle and water buffaloes (*Bubalus bubalis*) in the People's Republic of China. Veterinary Parasitology; 143(1): 79-85.

Zarkovic A, MacMurray C, Deva N, Ghosh S, Whitley D, Guest S. (2007) Seropositivity rates for *Bartonella henselae*, *Toxocara canis* and *Toxoplasma gondii* in New Zealand blood donors. Clinical and Experimental Ophthalmology; 35(2): 131-134.

Zhao GH, Zhang MT, Lei LH, Shang CC, Cao DY, Tian TT, Li J, Xu JY, Yao YL, Chen DK, Zhu XQ. (2011) Seroprevalence of *Toxoplasma gondii* infection in dairy goats in Shaanxi Province, Northwestern China. Parasites and Vectors; 4: 4.

Zhou D-H, Zhao F-R, Lu P, Xia H-Y, Xu M-J, Yuan L-G, Yan C, Huang S-Y, Li S-J, Zhu X-Q. (2012) Seroprevalence of Toxoplasma gondii infection in dairy cattle in southern China. Parasites and Vectors; 5: 48.

Zhou DH, Liang R, Yin CC, Zhao FR, Yuan ZG, Lin RQ, Song HQ, Zhu XQ. (2010) Seroprevalence of *Toxoplasma gondii* in pigs from southern China. Journal of Parasitology; 96(3): 673-674.

Zou F-C, Sun X-T, Xie Y-J, Li B, Zhao G-H, Duan G, Zhu X-Q. (2009) Seroprevalence of *Toxoplasma gondii* in pigs in Southwestern China. Parasitology International; 58(3): 306-307.

8 APPENDIX 1: HAZARD AND FOOD

8.1 *Toxoplasma gondii*

T. gondii is an obligate intracellular protozoan parasite that belongs to the phylum Apicomplexa, subclass coccidia (Montoya and Liesenfeld, 2004). The organism is able to reproduce sexually in large numbers in the definitive host (members of the cat family). Cysts are shed with faeces of the reservoir definitive host. Excreted organisms are able to infect intermediate hosts such as warm-blooded animals (including humans) and birds. The intermediate hosts are often also major food sources for humans, including livestock providing red meat (Goldsmid *et al.*, 2003).

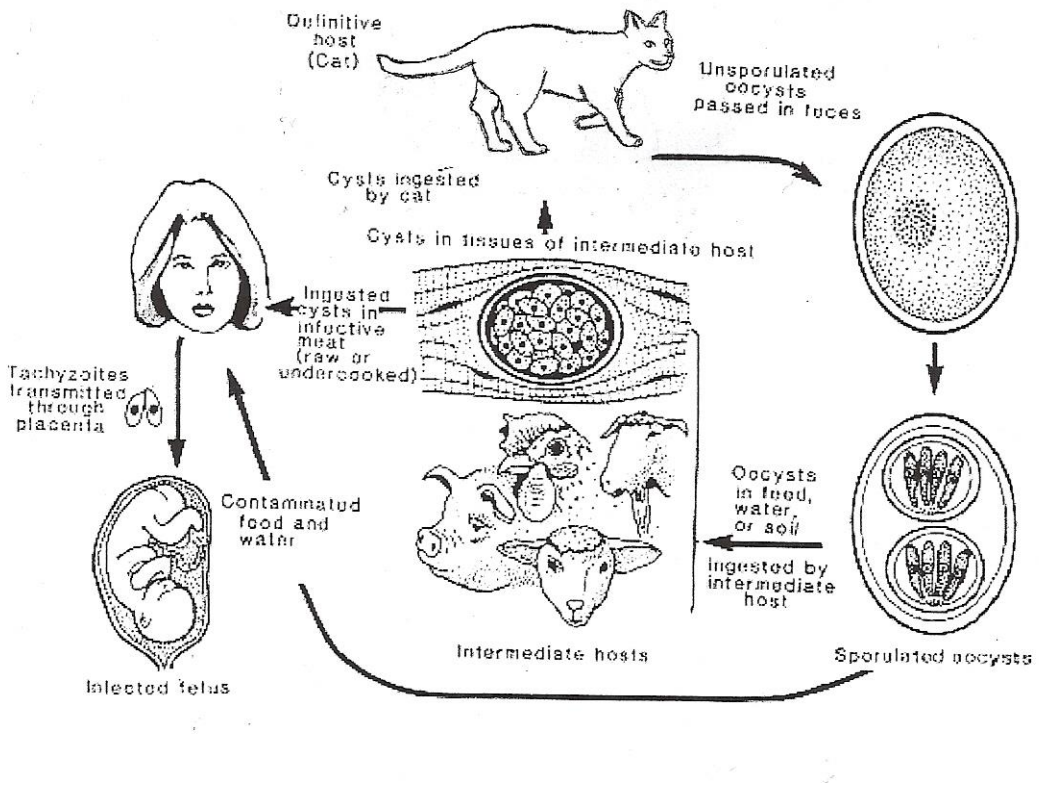
The organism has a complicated life cycle with numerous stages. The three infectious stages are tachyzoites, bradyzoites and sporozoites.

The environmentally resistant stage of *T. gondii* is called the oocyst, which are formed only in the cat family (*Felidae*). Cats can become infected and shed oocysts after eating any of the parasite's three lifestages (tachyzoite, bradyzoite or sporozoite), often in meat from intermediate hosts. The time to faecal shedding of oocysts depends on the lifestage ingested, for example 3 – 10 days for bradyzoites, 21 or more days for tachyzoites or sporozoites. The ingested bradyzoite appears better adapted to convert into oocysts in the cat's digestive system, with nearly 100% of cats shedding oocysts. However, after ingesting tachyzoites and sporozoites, fewer than 50% shed oocysts.

When mature, the oocysts are discharged into the intestine and passed out with the faeces. At this stage they are unsporulated and non-infectious. It takes one to five days (atmosphere and temperature dependent) to sporulate in the environment. Non-sporulated oocysts can survive in the environment for at least 3 months and still retain the ability to become infectious (Lindsay *et al.*, 2002). Mature oocysts contain two sporocysts, each sporocyst contains four sporozoites. Sporulation is necessary for the oocyst to be infective for the next host. The oocyst can remain infective in water or moist soil for over 12 months (Heymann, 2004). Estimates of the number of oocysts shed after initial infection ranges from 300,000 to 100 million, thereby logarithmically amplifying the cycle (Frenkel, 1990). A cat will usually have just one episode of shedding oocysts in its life and 90% of cats that have undergone the intestinal cycle will not shed oocysts again after re-infection with bradyzoites or cysts (Frenkel, 1990). Under experimental conditions, cats do not shed oocysts after reinoculation with *T. gondii* tissue cysts (bradyzoites), but this immunity can wane with time (Dubey, 1996).

Figure 1 depicts the life cycle of *T. gondii* from the cat as definitive host and how the organism is transmitted to humans via contaminated food and water and via undercooked meat.

Figure 1: **Diagram of the life cycle of T. gondii**



Source: (Dubey and Beattie, 1988)

The tachyzoite triggers a strong inflammatory response and tissue destruction, manifesting as clinical symptoms. Under pressure from the immune response, the tachyzoite forms intracellular cysts or bradyzoites in the tissue. The cyst wall is elastic, thin and can enclose hundreds of parasites (range 2 - >1000) (Dubey, 2001). Sites where the cysts are located can include visceral organs, such as lungs, liver and kidneys, although muscular and neural tissue is favoured such as the eye, brain, skeletal and cardiac muscle. Skeletal muscle tissues appears to be particularly effective in promoting the transition from tachyzoites to bradyzoites life stage (Ferreira-da-Silva *et al.*, 2009; Swierzy *et al.*, 2014). Intact, the cysts cause no harm and the bradyzoites can persist inside for the life of the host. However, if released from the cysts, bradyzoites transform into tachyzoites, are disseminated to all organs, and restart the infection (Montoya and Liesenfeld, 2004).

It has been theorised that humans could acquire and ingest oocysts from the fur of an infected cat. However, even where cats are passing faeces containing thousands of oocysts, none have been detected on the fur (Dubey, 1995).

General information on the growth, survival and inactivation of *T. gondii* is presented in the microbiological datasheet available from:
http://www.foodsafety.govt.nz/science-risk/hazard-data-sheets/pathogen-data-sheets.htm

T. gondii infection in humans may result from ingestion of oocyst-contaminated food or water, direct contact with cats or oocyst-contaminated soil, or from consumption of tissue cysts

present in meat (Dubey, 1994). Sporulation of oocysts results in release of sporozoites, while disruption of tissue cysts releases bradyzoites.

8.1.1 The Disease

Clinical toxoplasmosis typically affects individuals with developing or impaired immune systems such as the developing foetus, the elderly, medically immunosuppressed patients, and those who are immunocompromised by disease (e.g. AIDS), but may also occur as a mild disease in immunocompetent individuals (Smith, 1997). The central nervous system is the site most typically affected by infection, with a wide variety of symptoms (Montoya and Liesenfeld, 2004). Myocarditis has also been reported in the acute phase of *T. gondii* infection in both immunocompromised and immunocompetent individuals (Brownback *et al.*, 2012).

It has been reported that pregnant women are significantly (2.2 times) at higher risk of seroconversion (a change of status from seronegative to seropositive) for toxoplasmosis than non-pregnant women (7.7 times higher if the woman is an adolescent) (Avelino *et al.*, 2003). This has been ascribed to alterations in the immune system necessary for the tolerance of the foetus and/or hormonal imbalances (Daunter, 1992).

8.1.1.1 Congenital toxoplasmosis

If a previously unexposed pregnant woman becomes infected, the parasite can be transmitted across the placenta to cause congenital toxoplasmosis, the most commonly cited health concern. The mother may remain asymptomatic (Goldsmid *et al.*, 2003). In general, the older the mother, the more likely they are to be seropositive which means that those entering motherhood early may have more risk of initial infection while carrying their child. Women who are seropositive for *T. gondii* prior to pregnancy, but who are healthy and immunocompetent, do not transmit the parasite to their foetuses.

Transplacental infection occurs when rapidly dividing tachyzoite cells circulate in the bloodstream and cross the placenta to infect the foetus (Heymann, 2004). While the infection may not damage the foetus, it is capable of causing death or serious neurological damage in up to 5% of infections (Gilbert *et al.*, 2006). Hydrocephalus, intracranial calcification and chorioretinitis are the most common manifestations of tissue damage due to congenital toxoplasmosis (Gras *et al.*, 2001).

A 20-year prospective study (1985-2005) was carried out in Toulouse, France (Berrébi *et al.*, 2010). Maternal toxoplasmosis infections (n = 676) acquired during pregnancy were treated with spiramycin, alone or associated with pyrimethamine-sulfadoxine. Of 666 live born children, 112 (17%) had congenital toxoplasmosis. Of these children, 107 were followed for up to 21 years. The majority (n = 70; 74%) were symptomatic, while 28 (26%) developed chorioretinitis. Chorioretinitis usually occurred before age 5 years and always before 10 years of age. Visual impairment was usually not severe. More serious neurological problems (epilepsy) developed in one child.

8.1.1.2 Acute toxoplasmosis

In immunocompetent people, infection is asymptomatic in about 80% of cases (EFSA, 2007). Usually, clinical symptoms occur 10-14 days after infection and consist primarily of mild, local to generalised, self-limiting lymphadenopathy. Lymphadenopathy is seen in 3-20% of the

acutely infected people. Life-threatening cases of pneumonia due to *T. gondii* infection, have recently been described in immunocompetent individuals from South America and may be associated with genetically atypical and highly virulent strains of the parasite (Leal *et al.*, 2007).

A study in the USA, used acute toxoplasmic lymphadenopathy as an indicator of onset acute toxoplasmosis and investigated the uniformity of occurrence per month (Contopoulos-Ioannidis *et al.*, 2014). A peak in cases occurred in December (mid-Winter), with a second peak in September (early-Autumn). Similar temporal trends were observed in studies in Austria (Sagel *et al.*, 2010) and France (Morin *et al.*, 2012). These studies suggested that the increased consumption of contaminated fruit and vegetables in late-Summer and early-Autumn may have contributed to the observed Autumn peak.

8.1.1.3 Ocular toxoplasmosis

Ocular lesions may occur in association with congenital or acquired toxoplasmosis (Maenz *et al.*, 2014). Distinct regional differences are apparent, with particularly high prevalence reported in parts of South America, Africa and Asia. Although congenital toxoplasmosis frequently results in ocular toxoplasmosis, most cases are acquired after birth. Necrotising chorioretinitis is the typical presentation of ocular toxoplasmosis. Lesions may heal without impacting on vision or may cause extensive necrotic damage leading to legal blindness.

There is some evidence to suggest the congenital toxoplasmosis is more likely to result in lesions in the central region of the eye and to be bilateral (affecting both eyes) (Maenz *et al.*, 2014). Lesions in acquired toxoplasmosis are more likely to be randomly distributed and unilateral. Resultant legal blindness is more commonly associated with congenital toxoplasmosis.

In a study in Lyon, France a cohort of children with congenital toxoplasmosis (n = 477) were followed from birth until adolescence (Wallon *et al.*, 2014). At least one ocular lesion was observed in 142 cases (29.8%). The lesion were unilateral in 98 (69.0%) cases. There was no loss of vision in 80.6% of cases with unilateral lesions, where this could assessed. For assessed cases with bilateral lesions, vision was normal in 72.6% of cases. Severe bilateral visual impairment was not observed in any case.

8.1.1.4 People with HIV/AIDS

In immunocompromised people, particularly those with HIV/AIDS, disease seems to result from the activation of a previously subclinical infection. Reactivation most often involves the central nervous system and symptoms can include meningoencephalitis, maculopapular rash, generalised skeletal muscle involvement, cerebritis, chorioretinitis, pneumonia and myocarditis (Heymann, 2004). It has previously been estimated that up to 30% of AIDS patients in Europe and 10% in the USA died from toxoplasmosis, the majority via toxoplasmic encephalitis (Luft and Remington, 1992).

The introduction of anti-retroviral therapy (ART) appears to have had a significant impact on the burden of opportunistic infections in people with HIV/AIDS. A recent systematic review concluded that incidence rates of cerebral toxoplasmosis in people with HIV had decreased to be 1.2 to 8.0 times lower in the post-ART period compared to the pre-ART period (Coelho *et*

al., 2014). While most studies reviewed were conducted in North America and Europe, it appears that this trend is general.

8.1.2 Genotyping

Typing of *T. gondii* strains by polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) has resulted in greater discrimination of genetic variants of *T. gondii*. Typing is usually carried out at 6-11 gene loci. For each locus, an allelic PCR-RFLP pattern has been characterised, relating to each of the three lineages (I, II and III) (Su *et al.*, 2006). In isolates from Europe and North America, allelic variants associated with the same lineage will usually be found at each locus. However, genotyping of strains from other geographical regions and animal species have revealed isolates with mixtures of lineage I, II and III alleles, as well as alleles not aligned with the lineage classification (Bezerra *et al.*, 2012a; Dubey *et al.*, 2002; Dubey *et al.*, 2014a; Su *et al.*, 2006).

8.1.3 Pathogenicity

T. gondii was considered to have low genetic diversity with only three genetic types; I, II and III, classified on the basis of antigens, isoenzymes, and restriction fragment length polymorphism (Hill and Dubey, 2003). Most strains isolated from patients with AIDS are Type II. Type I and II strains have been recorded in patients with congenital disease, whereas strains isolated from animals are mostly Type III (Howe and Sibley, 1995; Montoya and Liesenfeld, 2004; Nowakowska *et al.*, 2006).

However, it now appears that there is much greater genetic diversity in *T. gondii* strains than was previously thought (Dubey and Su, 2009). Analysis of porcine strains from Brazil demonstrated a mixture of type classifications within a strain, depending on the genetic marker used for assignment (Belfort-Neto *et al.*, 2007). Three of four strains contained markers for types I and III, while the fourth strain contained markers for all three types. Similar mixed types have been observed for *T. gondii* isolates from pigs, sheep and cattle (Berger-Schoch *et al.*, 2011b; Boughattas *et al.*, 2014).

A German study compared *T. gondii* serotypes between cases with ocular toxoplasmosis and seropositive cases without ocular toxoplasmosis (Shobab *et al.*, 2013). A novel non-reactive serotype was found in 44% of ocular toxoplasmosis cases (n = 114), while the serotype was only found in 7% of seropositive cases of non-infectious autoimmune posterior uveitis (n = 56). Serotypes in non-ocular toxoplasmosis cases were predominantly type II.

The pathogenic potential of atypical strains was also demonstrated in the case of a French woman, who had been immunised against toxoplasmosis before conception, but became reinfected during pregnancy (Elbez-Rubinstein *et al.*, 2009). The reinfection was considered to be due to consumption of imported horse meat. The organism was isolated from the newborn and when genotyped with microsatellite markers, it exhibited an atypical genotype, one which is very uncommon in Europe but had been described in South America. Studies in a mouse model demonstrated that acquired immunity to European strains of *T. gondii* did not necessarily confer immunity against atypical strains from other regions.

8.2 *T. gondii* in Animals, Red Meat and Meat Products Overseas

8.2.1 Test methods

Most of the information regarding the prevalence of *T. gondii* is derived from studies that measure antibodies against the organism present in the blood of humans and animals. A review carried out by Remington *et al*. (1995) has examined the range of antibody detection methods including the Sabin-Feldman dye test, indirect fluorescent antibody test (IFAT), complement fixation test (CFT), the modified agglutination test (MAT) and enzyme-linked immunosorbent assay (ELISA).

It has been suggested that the performance of these methods in detecting serum antibodies may be species dependent (Mainar-Jaime and Barberán, 2007). Studies in sheep showed sensitivity and specificity greater than 90% for the MAT and indirect ELISA methods (Mainar-Jaime and Barberán, 2007),[17] while earlier studies in pigs showed sensitivities of 80.6% and 71.5% for the MAT and indirect ELISA methods, respectively, and specificities of 89.5% and 85.5% for the same two methods (Georgiadis *et al.*, 2003)

To detect *T. gondii* in meat or tissue samples, extracts or tissue samples are used in mouse-based assays to demonstrate infectivity. The mice are inoculated orally or subcutaneously with the extracts, and then the mouse sera can be examined for antibodies, and/or tissues can be examined microscopically. Assays have also been used with cats and, because of the large numbers of oocysts they shed following infection, they are more sensitive than mice for detecting infective cysts (Dubey, 2001). However, the mouse bioassay is more commonly used.

Results from such mouse assays have not consistently demonstrated that material extracted from the tissue of seropositive animals is infective, although it appears that material from seropositive animals with higher titres are more likely to be positive in the mouse bioassay. Heart tissues from 46 seronegative and 66 seropositive goats were digested with acid pepsin and the digest was inoculated into mice (Dubey *et al.*, 2011). *T. gondii* infections were determined in mice inoculated with heart material from one of 46 seronegative goats, one of 9 goats with titres of 1:10, one of 3 goats with titres of 1:40 and 26 of 40 goats with titres of 1:160 or greater.

Tissue cysts can also be detected directly by separation from macerated tissue by flotation or density separation (percoll) followed by light microscopy. Flow cytometry techniques have also been developed for the rapid enumeration of tissue cysts (Aldebert *et al.*, 2011).

The presence of *T. gondii* DNA in samples (including commercial meats) has been demonstrated using the polymerase chain reaction (PCR) (Aspinall *et al.*, 2002; Gutierrez *et al.*, 2010). PCR detects the presence of *T. gondii* DNA, but not necessarily the presence of viable cysts. A magnetic-capture PCR method has been reported to produce results in good agreement to the mouse bioassay, although the number of comparison samples was very small (n = 6) (Opsteegh *et al.*, 2010a).

[17] In direct ELISA assays a labelled antibody binds to the antigen (the molecule to be measured). In indirect ELISA assays, an unlabelled antibody binds to the antigen, then a labelled secondary antibody binds to the primary antibody

Techniques targeting *T. gondii* RNA have also been developed (Qu *et al.*, 2013). The method was reported to demonstrate good sensitivity and specificity relative to an RT-PCR technique, but was not compared to antibody or bioassay techniques.

T. gondii infection in experimentally and naturally infected pigs (*n* = 39) was confirmed by cat bioassay (Hill *et al.*, 2006). Serum ELISA was shown to be the most sensitive method for detecting *T. gondii* infected animals, followed by serum MAT, tissue fluid ELISA and two PCR methods. However, all of these methods also had marked rates of false positive detection. All methods performed better in experimentally infected animals, presumably due to higher concentrations of cysts. A seroprevalence study in sheep compared four methods (MAT, IFAT, ELISA and dye test) and concluded that the MAT test had the highest sensitivity, relative to the dye test, while the IFAT had the highest specificity (Shaapan *et al.*, 2008).

Mouse bioassays (4 mice per sample) were carried out on 22 blood samples that tested positive by serum MAT (Klun *et al.*, 2011). Cysts were found in the brains of 18 mice from 12 blood samples (54.5%).

8.2.2 Prevalence in red meat and meat products

A small number of studies have looked at the prevalence of *T. gondii* infectivity in red meat tissue or meat products. Results of studies published since the previous version of this Risk Profile are summarised in Table 5.

Table 5: Reported prevalence of *T gondii* in animal tissue

Country	No. tested	Meat tested	% positive	Assay Method	Reference
Brazil (North-East)	190	Pig brain, heart and tongue tissue	6.8	Pigs were screened by serology and seropositive animals (*n* = 37) assessed by mouse bioassay	(Feitosa *et al.*, 2014)
Brazil (São Paulo state)	602	Sheep brain, lung and muscle tissue	3.3[1] 3.7	Mouse bioassay PCR	(Silva *et al.*, 2011)
China (Anhui province)	416	Fresh pork	10.1 18.0 0.2[2]	Tissue fluid ELISA PCR Mouse bioassay	(Wang *et al.*, 2012)
Costa Rica	50 50	Ground meat Chorizo	4.0 4.0	Mouse bioassay	(Varela-Villalobos *et al.*, 2013)
Egypt	280	Sheep blood Sheep diaphragm tissue	50.4 61.4 10.0	LAT, cutoff 1:64 dilution ELISA Microscopy, mouse/kitten bioassay	(Hassanain *et al.*, 2011)
Ethiopia	47 44	Sheep hearts Goat hearts -Hearts were from seropositive animals	57.4 45.5	Mouse bioassay	(Gebremedhin *et al.*, 2014b)

Country	No. tested	Meat tested	% positive	Assay Method	Reference
France	426 -343 -83 374 -276 -98	Sheep diaphragms/hearts Domestic -Lambs -Adult sheep Imported -Lambs -Adult sheep	20 (5.4) 15 (2.0) 81 (42) 22 15 79	MAT, cutoff 1:6 dilution (mouse bioassay)	(Halos *et al.*, 2010)
France	419	Sheep hearts	11.5	Mouse bioassay	(Villena *et al.*, 2012)
Iran (South-East)	50	Fermented sausage	12	PCR	(Azizi *et al.*, 2014)
Iran (Ahvaz)	50 50 90	Beef Lamb Meat products (sausages, hamburgers, salami)	4.0 14.0 0.0	PCR	(Rahdar *et al.*, 2012)
Iran	48 46 40 30	Salami Sausage Hamburger Kebab	16.7 19.6 15.0 56.7	PCR	(Fallah *et al.*, 2011)
Mexico	48	Pork meat	2.1	Mouse bioassay	(Galván-Ramirez *et al.*, 2010)
Poland	145[3]	Meat (pork, beef, mutton, chicken) and meat products (sausages, salami, meat spread)	12.4	PCR	(Lass *et al.*, 2009)
Spain	475	Commercial Serrano ham	8.8 4.8	PCR Mouse bioassay	(Gomez-Samblas *et al.*, 2015)
Spain	50	Fresh pork meat (25) Commercial cured ham (25)	8 0	Mouse bioassay	(Bayarri *et al.*, 2012)
Switzerland	270 150 406 250	Diaphragm meat from: Pigs Wild boar Cattle Sheep	2.2 0.7 4.7 2.0	PCR of genomic DNA	(Berger-Schoch *et al.*, 2011b)
Tunisia	54	Sheep hearts	63 (serology) 31 (PCR)	MAT, cutoff 1:20 dilution PCR	(Boughattas *et al.*, 2014)
Turkey	50 50 120 20 100	Bovine brain Bovine muscle Ovine brain Ovine muscle Fermented sausage	2.0 6.0 4.2 20.0 19.0	Nested PCR	(Ergin *et al.*, 2009)
USA	33	Pig hearts (organic production)	51.5	Mouse bioassay	(Dubey *et al.*, 2012a)

NS = Not Stated; PCR = polymerase chain reaction; MAT = modified agglutination test; LAT = latex agglutination test; DNA = deoxyribose nucleic acids
*PCR methodology detects presence of *T. gondii* DNA, not the presence of viable cysts

[1] Only the 66 seropositive sheep were tested for the presence of infectivity or DNA in tissues. Of these 20 samples were infective, while 22 contained detectable DNA
[2] Only samples that were positive by both tissue fluid ELISA and PCR (n = 14) were analysed by mouse bioassay. Only one sample was positive by mouse bioassay
[3] The study included chicken, in addition to red meat type. Insufficient information was included to adjust sample number to only red meat

PCR tests for *T. gondii* DNA were positive for 8% of pork samples, 5% of beef, 4% of mutton and 2% of pig liver samples purchased from supermarkets in Taipei, Taiwan (Fuh *et al.*, 2013). However, all samples tested negative for IgG antibodies to *T. gondii* and microscopic examination of PCR-positive samples did not identify any *T. gondii* cysts. Overall, 88% of sheep sampled were seropositive for *T. gondii* by IFAT (cutoff 1:16 dilution). Tissue cysts were significantly more prevalent in animals with high antibody titres. Infectivity of cysts was not assessed by bioassay. The prevalence of tissue cysts decreased with animal age, with the highest prevalence in animals less than one year old.

Several direct and indirect methods were compared for detection of *T. gondii* in tissues (brain and tongue) of pigs (n = 20) (Bezerra *et al.*, 2012b). Direct PCR of pig tissues detected *T. gondii* DNA in 10% of samples. The remainder of the tissues were processed and inoculated into mice (indirect method). Mice were euthanised after 42 days. PCR of mouse tissues indicated that 55% of pigs were infected with *T. gondii*, while examination of brain and lung tissue slides identified cysts in mice related to 25% of pigs. Immunohistochemical analysis of mouse tissues indicated a pig positivity of 30%, while histopathology of mouse tissues did not detect the parasite.

Tissue cysts were recovered from sheep brain (36/100) and skeletal muscle (32/100) tissue by Percoll (density) gradient centrifugation (Yildiz *et al.*, 2014). Nested PCR was used to confirm *T. gondii* DNA in the isolated cysts.

Very few quantitative data are available for concentrations of *T. gondii* on meat and meat products. A study of contamination of commercial Serrano ham provided quantitative estimates of *T. gondii* tachyzoites by quantitative real-time PCR (qRT-PCR) (Gomez-Samblas *et al.*, 2015). The average *T. gondii* tachyzoites concentration in ham pieces that were positive by PCR and mouse bioassay was reported to be 2.27×10^5 tachyzoites per 100 g of ham, while the average concentration in ham slices was 5.56×10^4 per 100 g of ham.

Skeletal muscle cells are considered to be a preferred cell type for parasite persistence and tissue cyst formation (Swierzy *et al.*, 2014). There is evidence to suggest that this is due to cell cycle regulators involved in formation of muscle fibres (myotubes) directly or indirectly promoting *T. gondii* stage conversion.

8.2.3 Seroprevalence in animals

The prevalence of antibodies for *T. gondii* in animals overseas derived from serological tests has been summarised in Table 6. Information has only been collated for species that fall within the scope of the current Risk Profile (cattle, sheep, deer, goats, pigs and water buffalo).

While infections in animals are assumed to occur through environmental contact with sporulating oocysts, vertical transmission of infections from ewes (Buxton *et al.*, 2006), cows (Garcia *et al.*, 2012) and sows (García-Bocanegra *et al.*, 2010c) to their foetuses or young has been investigated. While antibodies for *T. gondii* have been detected in foetuses and newborn animals, no evidence of infection has been demonstrated. A study in pigs demonstrated that only piglets from seropositive sows were seropositive at one week of age (García-Bocanegra *et al.*, 2010c). The level of maternally-derived antibodies decreased from birth, with the longest duration of seropositivity associated with piglets from sows with the highest antibody titres. Seropositivity due to horizontal transfer of infection increased with increasing animal age.

Studies using PCR detection of the SGA1 gene of *T. gondii* indicated that vertical transmission of infection occurred in 66% of sheep pregnancies (Hide *et al.*, 2009). It should be noted that neither the *T. gondii* status of the ewes, nor the presence of clinical symptoms in the lambs, was reported and the significance of the presence of *T. gondii* genetic material in lambs requires further investigation. It was further reported that similar methodology indicated congenital transmission of *T. gondii* in 75% of mouse pregnancies and 19.8% of human pregnancies.

8.2.4 Recalls

No information on recalls of red meat or meat products due to the presence of *T. gondii* was found. This is not surprising considering that testing of these products for viable *T. gondii* is very specialised.

Table 6: Reported prevalence of *T. gondii* antibodies in overseas animals

Country	Number tested	Tests performed	Seropositive (%)	Risk factors identified/Comments	Reference
Cattle					
Bangladesh (subsistence farms)	37	LAT, cutoff 1:32 dilution	27.0		(Rahman *et al.*, 2014)
Bangladesh (Mymensingh district)	25	LAT, cutoff 1:32 dilution	12.0		(Shahiduzzaman *et al.*, 2011)
Brazil (Rio de Janeiro state)	589	IFAT, cutoff 1:64 dilution	14.8	Number of cats Cats in contact with cattle Cats in contact with drinking water	(Albuquerque *et al.*, 2011)
Brazil (Rio de Janeiro state, north)	77	ELISA	49.4		(Frazão-Teixeira and de Oliveira, 2011)
Brazil (Rio de Janeiro state)	459	IFAT, cutoff 1:64 dilution	2.0		(Luciano *et al.*, 2011a)
Brazil (Rio Grande do Sul state)	121	IFAT, cutoff 1:64 dilution	17.4		(de F. Santos *et al.*, 2013)
Brazil (Minas Gérais state)	1195	IFAT, cutoff 1:64 dilution	2.7	Semi-intensive rearing system Feed stored on farm Veterinarian examinations not performed History of abortions or stillbirths Number of cats Greater number of different animal species on farm	(Fajardo *et al.*, 2013)
Brazil (South)	120	IFAT, cutoff 1:50 dilution	Dairy 29.1	Animal pregnant Breed	(Silveira Barbosa de Macedo *et al.*, 2012)
Brazil (Paraná state)	169 cows 81 foetuses	IFAT, cutoff 1:50 dilution (cows), 1:25 (foetuses)	26.0 2.5		(Garcia *et al.*, 2012)
Brazil (Pernambuco state)	427	IFAT, cutoff 1:64 dilution	16.6		(Guerra *et al.*, 2014)
Brazil (Mato Grosso state)	2000	IFAT, cutoff 1:64 dilution	Dairy cows 71.0		(Santos *et al.*, 2009)

Country	Number tested	Tests performed	Seropositive (%)	Risk factors identified/Comments	Reference
Brazil (Guarapuava city)	250	IFAT, cutoff 1:64 dilution	30.8 (56.0% farm prevalence)	Animal age (higher prevalence in older animals) Mixed-breed animals compared to pure-breed	(de Moura *et al.*, 2010)
Brazil (Bahia state)	600	IFAT, cutoff 1:64 dilution	11.8		(Spagnol *et al.*, 2009)
China	262 Dairy 10 Beef	IHAT	2.3 Dairy 0.0 Beef		(Yu *et al.*, 2007)
China (North-East)	1110 Dairy 693 Beef	IHAT	2.3 Dairy 3.0 Beef		(Qiu *et al.*, 2012)
China (Guangzhou)	350	IHAT, cutoff 1:64 dilution	Dairy cows 5.7		(Zhou *et al.*, 2012)
China (Liaoning province)	646	IHAT	6.0		(Liu *et al.*, 2012)
China (Jinzhou city)	350	IHAT, cutoff 1:64 dilution	Dairy 6.9	Fewer pregnancies	(Bao *et al.*, 2012)
China (Jilin province)	1040	ELISA	12.8		(Ge *et al.*, 2014)
China (Guangxi Zhuang Autonomous Region)	875	IHAT	Dairy 13.7		(Xu *et al.*, 2012)
France	1329	MAT, cutoff 1:24 dilution	7.8 (herd prevalence 87.5)	Small herd size Water source on pasture	(Gilot-Fromont *et al.*, 2009a)
India (Punjab)	83	ELISA	2.4		(Sharma *et al.*, 2008)
Iran (Fars province)	80	MAT, cutoff 1:20 dilution	55		(Asgari *et al.*, 2013)
Iran (Fars province)	588	IFAT, cutoff 1:16 dilution	20.2		(Asgari *et al.*, 2010)
Iran (South-East)	70	PCR of tongue, brain, femur muscle and liver samples	8.6		(Azizi *et al.*, 2014)
Iran (South-West)	450 (female only)	MAT, cutoff 1:25 dilution	15.8		(Hamidinejat *et al.*, 2010)
Iran (Isfahan and Chaharmahal va Bakhtiary provinces)	155	PCR of blood	0.0		(Khamesipour *et al.*, 2014)

Country	Number tested	Tests performed	Seropositive (%)	Risk factors identified/Comments	Reference
Japan	422	LAT, cutoff 1:64 dilution	7.3		(Matsuo *et al.*, 2014)
Korea	105	LAT	3.8		(Song *et al.*, 2011)
Malaysia	126	IFAT, cutoff 1:200 dilution	6.3		(Chandrawathani *et al.*, 2008)
Netherlands[1]	995	In-house ELISA	<8 months 0.5/1.9 8-12 months 5.9/15.6 >12 months 22.7/54.5		(Opsteegh *et al.*, 2011b)
New Caledonia	30	ELISA	3.3		(Roqueplo *et al.*, 2011)
Nigeria (Ibadan)	210	IgG by ELISA	13.9		(Onyiche and Ademola, 2013)
Pakistan	400	IgG and IgM by ELISA	IgG 18.8 IgM 2.3 Total 19.8	Poor hygienic practice Extensive (low input) management practice Presence of cats	(Ahmad and Qayyum, 2014)
Poland (North)	4033	IgG by ELISA	3.2 (37.9% herd prevalence)	Animal age (higher prevalence in older animals) Small herd size Traditional farming methods	(Holec-Gasior *et al.*, 2013)
Poland	865	MAT ELISA	12.8 14.6		(Sroka *et al.*, 2011)
Portugal	161	MAT, cutoff 1:100 dilution	7.5		(Lopes *et al.*, 2013)
Senegal	103	MAT	12.6		(Davoust *et al.*, 2015)
South Africa (North West)	178	ELISA	20.8		(Ndou *et al.*, 2013)
Spain (Galacia)	178	DAT, cutoff 1:64 dilution	7.3		(Panadero *et al.*, 2010)
Spain (Southern)	504	ELISA	83.3 (100% herd prevalence)		(García-Bocanegra *et al.*, 2013)
Sudan	181	ELISA	13.3	Samples were from herds that had experienced reproductive problems (abortions, infertility and stillbirths) Prevalence was significantly higher in animals <1 year than older animals	(Elfahal *et al.*, 2013)

Country	Number tested	Tests performed	Seropositive (%)	Risk factors identified/Comments	Reference
Switzerland	406	ELISA (meat juice)	45.6		(Berger-Schoch *et al.*, 2011a)
Tanzania (Tanga region)	655	LAT	3.6 (13.0% herd prevalence)	Traditional husbandry compared to smallholder practises Larger herd size	(Schoonman *et al.*, 2010)
Thailand	700	 LAT ELISA	Dairy 9.4 17.0		(Inpankaew *et al.*, 2010)
Thailand (western)	389	IFAT, cutoff 1:128 dilution	25.7		(Wiengcharoen *et al.*, 2012)
Turkey (Kirikkale province)	100	Sabin-Feldman dye test, cutoff 1:16 dilution	Dairy 53.0		(Ocal *et al.*, 2008)
West Indies (Grenada and Carriacou)	119	MAT, cutoff 1:25 dilution	8.4		(Chikweto *et al.*, 2011)
Sheep					
Argentina (Humid Pampa region)	704	IFAT, cutoff 1:50 dilution	Dairy 17.3 (100% flock prevalence)		(Hecker *et al.*, 2013)
Bangladesh (subsistence farms)	83	LAT, cutoff 1:32 dilution	69.9		(Rahman *et al.*, 2014)
Bangladesh (Mymensingh district)	25	LAT, cutoff 1:32 dilution	40.0		(Shahiduzzaman *et al.*, 2011)
Belgium	3170	IgG by ELISA	87.4 (96.2% flock prevalence)		(Verhelst *et al.*, 2014)
Brazil, North-East	930	IgG by ELISA	22.1	Running water source Presence of cats Adult animals, rather than lambs	(Andrade *et al.*, 2013)
Brazil (Pernambuco)	50	IFAT, cutoff 1:64 dilution	26.0	For one seropositive ewe genomic DNA was detected by PCR in reproductive tissues and the foetus	(Bezerra *et al.*, 2014)
Brazil (São Paulo State)	382	MAT and IFAT, cutoff 1:16 dilution	18.6		(Langoni *et al.*, 2011)
Brazil (São Paulo State)	602	MAT and IFAT, cutoff 1:16 dilution	11.0		(Silva *et al.*, 2011)

Country	Number tested	Tests performed	Seropositive (%)	Risk factors identified/Comments	Reference
Brazil (Jaboticabal microregion)	488	IFAT, cutoff 1:64 dilution	52.0	Gender (rams > ewes) Contact with cats Extensive production system No mineral supplementation Pasture/fresh bulk feed (compared to hay)	(Lopes *et al.*, 2010)
Brazil (Minas Gérais state)	711	ELISA IFAT	31.1 43.2	Animal age (higher prevalence in older animals)	(Carneiro *et al.*, 2009)
Brazil (Alagoas state)	NS	IFAT	32.9	Animal age (higher prevalence in older animals) Smaller property size Semi-intensive rearing system Running water source Presence of cats	(Pinheiro *et al.*, 2009)
Brazil (Federal District)	1028	IFAT, cutoff 1:64 dilution	38.2		(Ueno *et al.*, 2009)
Brazil (Rio de Janeiro state)	379	MAT, cutoff 1:25 dilution	53.3		(Cosendey-KezenLeite *et al.*, 2014)
Brazil (Rio de Janeiro state)	360	IFAT, cutoff 1:64 dilution	38.1		(Luciano *et al.*, 2011b)
Brazil (Santa Catarina state)	360	IFAT, cutoff 1:64 dilution ELISA	56.9 42.5	Breed	(Liz Stefen Sakata *et al.*, 2012)
Brazil (Rio Grande do Norte state_	102	IgG by ELISA	29.4	Animal age (higher prevalence in older animals)	(Clementino *et al.*, 2007)
Caribbean - Dominica - Grenada - Monserrat - St Kitts and Nevis	 55 84 28 138	ELISA	 67.3 47.6 89.3 56.5		(Hamilton *et al.*, 2014)
China (Heilongjiang province)	792	IHAT, cutoff 1:64 dilution	3.0		(Wang *et al.*, 2011)
China (Tibet)	455	IHAT, cutoff 1:64 dilution	Tibetan sheep 5.7		(Wu *et al.*, 2011)
China (Liaoning province)	566	IHAT, cutoff 1:64 dilution	4.4		(Yang *et al.*, 2013)

Country	Number tested	Tests performed	Seropositive (%)	Risk factors identified/Comments	Reference
Czech Republic	547	IgG by ELISA	59.4		(Bártová *et al.*, 2009)
Egypt	300	Dye test MAT, 1:25 cutoff IFAT, 1:64 cutoff ELISA	34.0 43.7 36.7 41.7		(Shaapan *et al.*, 2008)
Egypt (El Fayoum governorate)	62	IgG by ELISA PCR	98.4 67.7		(Ghoneim *et al.*, 2009)
Ethiopia	1130	IgG by ELISA	31.6 (70.5% flock prevalence)	Higher altitude of farm Female animals Animal age Small flocks Water source	(Gebremedhin *et al.*, 2013)
Ethiopia (Central)	305	DAT, cutoff 1:40 dilution	20.0	Age Season	(Gebremedhin *et al.*, 2014a)
Finland	1940	DAT, cutoff 1:40 dilution	24.6 (76.3% flock prevalence)		(Jokelainen *et al.*, 2010)
France	419	MAT, cutoff 1:4 dilution ELISA	24.8 16.9		(Villena *et al.*, 2012)
Greece	458	IgG by ELISA	Dairy sheep 53.7		(Anastasia *et al.*, 2013)
Greece (Northern)	1501	ELISA	48.6	Intensive or semi-intensive production Feeding concentrate Using water from a public supply	(Tzanidakis *et al.*, 2012)
India (Punjab)	186	ELISA	3.8		(Sharma *et al.*, 2008)
Iran (Fars Province)	95	MAT, cutoff 1:20 dilution	29.5		(Asgari *et al.*, 2013)
Iran (South-East)	50	PCR of tongue, brain, femur muscle and liver samples	38	Infection rate was correlated with animal age	(Azizi *et al.*, 2014)
Iran (Isfahan and Chaharmahal va Bakhtiary provinces)	95	PCR of blood	17.9		(Khamesipour *et al.*, 2014)
Iran (Babol region)	285	IFAT, cutoff 1:100 dilution	31.2		(Youssefi *et al.*, 2007)

Country	Number tested	Tests performed	Seropositive (%)	Risk factors identified/Comments	Reference
Iran (Kerman)	90	IFAT, cutoff 1:16 dilution	3.3		(Derakhshan and Mousavi, 2014)
Ireland	292	LAT, cutoff 1:64 dilution	36.0		(Halová *et al.*, 2013)
Italy (Grosseto district, Tuscany)	630	IFAT, cutoff 1:64 dilution	Dairy sheep 34.0 (97.0% flock prevalence)	Stray cats with access to animals' water Small flock size Still water source	(Cenci-Goga *et al.*, 2013; Sechi *et al.*, 2013)
Italy (Campania)	1170	IFAT, cutoff 1:200 dilution	28.5		(Fusco *et al.*, 2007)
Italy (Sicily)	1961	IgG by ELISA	49.9 (87.1% farm prevalence)	Cats on farm Use of surface water source Greater farm size Age of animals (higher prevalence in older animals)	(Vesco *et al.*, 2007)
Japan	267	IFAT, cutoff 1:40 dilution	28.8		(Giangaspero *et al.*, 2013)
Lithuania	354	ELISA	42.1		(Stimbirys *et al.*, 2007)
Mexico	405	MAT, cutoff 1:25 dilution	29.9	Higher farm altitude Lower mean temperature Lower mean annual rainfall	(Alvarado-Esquivel *et al.*, 2013d)
Mexico (Durango state)	511	MAT, cutoff 1:25 dilution	15.1	Age of animals (higher prevalence in older animals)	(Alvarado-Esquivel *et al.*, 2012b)
Mexico (Oaxaca state)	429	MAT, cutoff 1:25 dilution	23.1	Semi-intensive management compared to semi-extensive Mixed-breed sheep compared to pure-breed Higher altitude	(Alvarado-Esquivel *et al.*, 2013b)
Netherlands	1179	In-house ELISA	27.8	Age of animals (higher prevalence in older animals) Animals from central region	(Opsteegh *et al.*, 2010b)
Poland	41	IFAT, cutoff 1:8 dilution	IgG 53.7 IgM 0.0	Seropositive sheep were found to enter the milking parlour significantly later than seronegative sheep	(Górecki *et al.*, 2008)
Portugal	119	MAT, cutoff 1:20 dilution	33.6	Age of animals (higher prevalence in older animals)	(Lopes *et al.*, 2013)
Saudi Arabia	30	LAT, IHAT	33.3-46.7		(Eisa *et al.*, 2013)

Country	Number tested	Tests performed	Seropositive (%)	Risk factors identified/Comments	Reference
Saudi Arabia (Riyadh)	891	IFAT, cutoff 1:32 dilution	36.4		(Alanazi, 2013)
Scotland	3333	IgG by ELISA	56.6 (100% flock prevalence)	Age of animals (higher prevalence in older animals) Lambing on paddocks/parks Grazing on common land (contact with sheep from other farms) Farms with multiple boundaries	(Katzer *et al.*, 2011)
Senegal	43	MAT	16.3		(Davoust *et al.*, 2015)
South Africa	600	IFAT ELISA	5.6 4.3	Higher temperatures Commercial farms compared to informal farming Intensive or semi-intensive management	(Samra *et al.*, 2007)
Spain (North-West)	2400	DAT Indirect ELISA	38.1 (100% flock prevalence)	Age of animals (higher prevalence in older animals) Coastal location of farm	(Díaz *et al.*, 2014)
Spain (Galacia)	177	DAT, cutoff 1:64 dilution	57.1		(Panadero *et al.*, 2010)
Spain (Southern)	503	ELISA	49.3 (84.7% flock prevalence)		(García-Bocanegra *et al.*, 2013)
Switzerland	250	ELISA (meat juice)	61.6		(Berger-Schoch *et al.*, 2011a)
Tunisia	Lambs 217 Ewes 125	MAT, cutoff 1:20 dilution	38.2 73.6		(Boughattas *et al.*, 2014)
UK	Neonatal lambs: Charollais 243 Swaledale 264 4-month lambs: Charollais 411 Swaledale 329	PCR MAT, cutoff 1:25 dilution	 6.6 11.4 12.2 3.0		(Mason *et al.*, 2010)
UK	3539	LAT	74.0	Age of animals (higher prevalence in older animals) Cattle on the same farm	(Hutchinson *et al.*, 2011)
USA (lambs)	383	MAT, cutoff 1:25 dilution	27.1	*T. gondii* was isolated from 53/68 hearts from seropositive lambs by cat or mouse bioassay	(Dubey *et al.*, 2008)

Country	Number tested	Tests performed	Seropositive (%)	Risk factors identified/Comments	Reference
USA (lambs)	3967	MAT	9.4 (47.3% flock prevalence)		(APHIS, 2014)
West Indies (Grenada and Carriacou)	204	MAT, cutoff 1:25 dilution	44.1		(Chikweto *et al.*, 2011)
Water Buffalo					
Brazil (Rio Grande do Sul state)	169	IFAT, cutoff 1:64 dilution	27.2		(de F. Santos *et al.*, 2013)
China	40	IHAT	0.0		(Yu *et al.*, 2007)
India (Punjab)	103	ELISA	2.9		(Sharma *et al.*, 2008)
Pakistan	422	IgG and IgM by ELISA	IgG 13.7 IgM 2.4 Total 15.2	Poor hygienic practice Extensive (low input) management practice Presence of cats	(Ahmad and Qayyum, 2014)
Goats					
Bangladesh (subsistence farms)	146	LAT, cutoff 1:32 dilution	61.0	Age of animals (higher prevalence in older animals)	(Rahman *et al.*, 2014)
Bangladesh (Mymensingh district)	25	LAT, cutoff 1:32 dilution	32.0		(Shahiduzzaman *et al.*, 2011)
Brazil (Ceará)	2362	ELISA	25.1	Use of wooden feeding troughs or absence of feeding troughs Age of animals (higher prevalence in older animals) More than 10 cats on farm	(Cavalcante *et al.*, 2008)
Brazil (Paraiba state)	975	IFAT, cutoff 1:64 dilution	Dairy 18.1 (70% herd prevalence)	Presence of toxic plants Goat breeding not the main activity on the farm	(Santos *et al.*, 2012)
Brazil (Mossoro)	338	ELISA	37.0		(Nunes *et al.*, 2013)
Brazil (Rio de Janeiro state)	206	IFAT, cutoff 1:64 dilution	29.1		(Luciano *et al.*, 2011b)
Caribbean - Dominica - Grenada - Monserrat - St Kitts and Nevis	 136 94 31 181	ELISA	 58.1 57.4 80.1 42.0		(Hamilton *et al.*, 2014)

Country	Number tested	Tests performed	Seropositive (%)	Risk factors identified/Comments	Reference
China (Heilongjiang province)	792	IHAT, cutoff 1:64 dilution	3.8		(Wang *et al.*, 2011)
China (Shaanxi province)	751	IHAT, cutoff 1:64 dilution	Dairy goats 14.1		(Zhao *et al.*, 2011)
Egypt (El Fayoum governorate)	24	IgG by ELISA PCR	41.7 25.0		(Ghoneim *et al.*, 2009)
Ethiopia (Central)	927	IgG by ELISA	19.7 (58.3% herd prevalence)	Semi-intensive management compared to extensive management Female animals Age of animals (higher prevalence in older animals) Small herd size Drinking water from tap Sedentary or agro-pastoral farm system	(Gebremedhin *et al.*, 2014a)
Ethiopia (Central)	323	DAT, cutoff 1:40 dilution	15.5		(Gebremedhin *et al.*, 2014a)
Greece	375	IgG by ELISA	Dairy goats 61.3		(Anastasia *et al.*, 2013)
Greece (Northern)	541	ELISA	30.7	Intensive or semi-intensive production Feeding concentrate Using water from a public supply	(Tzanidakis *et al.*, 2012)
Iran (Fars Province)	90	MAT, cutoff 1:20 dilution	18.8		(Asgari *et al.*, 2013)
Iran (Kerman)	114	IFAT, cutoff 1:16 dilution	1.7		(Derakhshan and Mousavi, 2014)
Italy	127	MAT, cutoff 1:20 dilution	60.6		(Mancianti *et al.*, 2013)
Malaysia	200	IFAT, cutoff 1:200 dilution	35.5		(Chandrawathani *et al.*, 2008)
Mexico (Michoacan state)	341	MAT, cutoff 1:25 dilution	Dairy 15.2		(Alvarado-Esquivel *et al.*, 2013c)
Norway	2188	DAT	Dairy 17.2 (75.3% herd prevalence)		(Stormoen *et al.*, 2012)
Portugal	184	MAT, cutoff 1:20 dilution	18.5	Age of animals (higher prevalence in older animals)	(Lopes *et al.*, 2013)

Country	Number tested	Tests performed	Seropositive (%)	Risk factors identified/Comments	Reference
Romania (dairy)	735	ELISA	52.8	Age of animals (higher prevalence in older animals) Backyard raising systems	(Iovu *et al.*, 2012)
Saudi Arabia	31	LAT, Indirect haemagglutination	29.0-45.2		(Eisa *et al.*, 2013)
Saudi Arabia (Riyadh)	555	IFAT, cutoff 1:32 dilution	35.3		(Alanazi, 2013)
Senegal	52	MAT	15.4		(Davoust *et al.*, 2015)
Serbia	431	MAT, cutoff 1:25 dilution	73.3	Outside access (compared to stable reared) Mixed usage (compared to dairy only) Feeding practices Water source Exclusive farming of goats	(Djokic *et al.*, 2014)
Spain (Southern)	494	ELISA	25.1 (72.2% herd prevalence)		(García-Bocanegra *et al.*, 2013)
Tanzania (Northern)	337	LAT, cutoff 1:16 dilution	19.3 (45.2% herd prevalence)		(Swai and Kaaya, 2012)
USA	75	MAT, cutoff 1:25 dilution	19.0		(Keith and Alex, 2010)
USA (Missouri)	367	LAT, cutoff 1:32 dilution	Boer goats 6.8 (41.7% herd prevalence)		(Yaglom *et al.*, 2014)
USA	234	MAT, cutoff 1:5 dilution	53.4		(Dubey *et al.*, 2011)
West Indies (Grenada and Carriacou)	180	MAT, cutoff 1:25 dilution	42.8		(Chikweto *et al.*, 2011)
Pigs					
Argentina	Outdoor (149) Indoor (148)	Study compared four methods – IFAT (cutoff 1:50 dilution), ELISA, Western blot and tachyzoite surface antigen ELISA	75.2-83.2 4.7-23.0		(Basso *et al.*, 2013)

Country	Number tested	Tests performed	Seropositive (%)	Risk factors identified/Comments	Reference
Austria	1368	MAT ELISA I ELISA II ELISA III	6.5 6.7 4.8 4.3		(Steinparzer *et al.*, 2015)
Brazil	50	PCR	Diaphragms 34 Tongues 66 Both 28		(Belfort-Neto *et al.*, 2007)
Brazil (Rio Grande do Sul)	100	IFAT, cutoff 1:64 dilution	36.0	*T. gondii* was isolated from tissues of 17/36 serology-positive pigs (47.2%). Infectivity was confirmed by mouse bioassay	(Cademartori *et al.*, 2014)
Brazil (Southern Piauí)	143	IgG by ELISA	25.5	Animals' age Feeding human food scraps Less intensive rearing systems Presence of cats on farms	(de Sousa *et al.*, 2014)
Brazil (Pernambuco state)	305	IFAT, cutoff 1:64 dilution	12.5		(Samico Fernandes *et al.*, 2012)
Brazil (North-East)	190	IFAT, cutoff 1:64 dilution	19.5	Extensive husbandry Feeding with leftovers	(Feitosa *et al.*, 2014)
Brazil (Mato Grosso)	708	IFAT, cutoff 1:64 dilution	12.8		(Muraro *et al.*, 2010)
Brazil (Rio de Janeiro state, north)	61	ELISA	11.5	Free-ranging pigs compared to indoor housed pigs	(Frazão-Teixeira and de Oliveira, 2011)
Brazil (Rio de Janeiro state)	406	IFAT, cutoff 1:64 dilution	7.6		(Luciano *et al.*, 2011a)
Canada (Ontario)	2848 (2001) 1600 (2003) 1600 (2004)	ELISA	1.59 0.06 0.26		(Poljak *et al.*, 2008)
Chile	340	NS	8.8		(Munoz-Zanzi *et al.*, 2012)
China (Hunan province)	1191	IHAT, cutoff 1:64 dilution	Sows 31.3		(Xu *et al.*, 2014)
China (Yunnan province)	831	IHAT, cutoff 1:64 dilution	17.0		(Zou *et al.*, 2009)

Country	Number tested	Tests performed	Seropositive (%)	Risk factors identified/Comments	Reference
China (Central)	3558	ELISA	24.5	Contact of pigs with cats High density of pig breeding Presence of mosquitoes and flies Low frequency of scavenging	(Tao *et al.*, 2011)
China (Liaoning province)	1164	IHAT	12.0		(Liu *et al.*, 2012)
China (Chongqing)	908	IHAT	30.6		(Wu *et al.*, 2012)
China (Jiangxi province)	1232	IHAT, cutoff 1:64 dilution	22.9		(Jiang *et al.*, 2014)
China (Heilongjiang province)	1014	IHAT	4.6	Small farm size	(Chang *et al.*, 2012)
China (Western Fujian province)	605	IHAT	Breeding sows 14.4		(Huang *et al.*, 2010)
China (Guangdong province)	1022	ELISA	27.0		(Zhou *et al.*, 2010)
Côte d'Ivoire	91	ELISA	8.8		(Prangé *et al.*, 2009)
Czech Republic	551	IgG by ELISA	35.9		(Bártová and Sedlák, 2011)
Germany	3323	Meat juice ELISA	9.9 (88% herd prevalence)		(Meemken *et al.*, 2014)
Ireland	317	LAT, cutoff 1:64 dilution	4.7		(Halová *et al.*, 2013)
Italy (Umbria)	960	IFAT, cutoff 1:16 dilution	16.1	All-in all-out housing Cleaning method	(Veronesi *et al.*, 2011)
Italy (Sicily)	3472	IgG by ELISA	Imported 0.7 Domestic 16.3	Domestic only: Farming type (highest in farrow-to-finish) Rodenticides not used Manual cleaning rather than semi-automatic cleaning Lower altitude Smaller herd size Water sourced from wells	(Villari *et al.*, 2009)
Japan	155	LAT, cutoff 1:64dilution	5.2		(Matsuo *et al.*, 2014)
Latvia	803 (Domestic) 606 (Wild boar)	In-house ELISA	4.2 33.2	Free-ranging	(Deksne and Kirjusina, 2013)

Country	Number tested	Tests performed	Seropositive (%)	Risk factors identified/Comments	Reference
Malaysia	100	IFAT, cutoff 1:200 dilution	0.0		(Chandrawathani *et al.*, 2008)
Mexico (Yucatan)	429	IgG by ELISA IgM by ELISA PCR	95.8 92.5 50.8	Small farm size Type of feeder	(Ortega-Pacheco *et al.*, 2013)
Mexico (Durango state)	1074	MAT, cutoff 1:25 dilution	12.7	Age of animals (higher prevalence in older animals) Raised in mountainous regions, rather than valleys or semi-desert Mixed-breed pigs compared to pure-breed Geographical location	(Alvarado-Esquivel *et al.*, 2011c)
Mexico (Veracruz state)	402	MAT, cutoff 1:25 dilution	45.3	Tropical-humid climate Feeding with leftovers Storing pig food in owner's house Free-ranging	(Alvarado-Esquivel *et al.*, 2014b)
Mexico (Oaxaca state)	337 (Backyard) 188 (Farm raised)	MAT, cutoff 1:25 dilution	17.2 0.5	Age of animals (higher prevalence in older animals) Greater altitude of raising environment Tropical climate	(Alvarado-Esquivel *et al.*, 2012a)
Nepal	742	ELISA	11.7		(Devleesschauwer *et al.*, 2013)
Netherlands	845	ELISA	2.6	Free-ranging > organic > intensive	(van der Giessen *et al.*, 2007)
New Caledonia	49	ELISA	2.0		(Roqueplo *et al.*, 2011)
Nigeria (Ibadan)	302	IgG by ELISA	29.1		(Onyiche and Ademola, 2013)
Panama	290	IFAT, cutoff 1:20 dilution	32.1		(Correa *et al.*, 2008)
Poland	1754	IgG by ELISA	19.2		(Holec-Gasior *et al.*, 2010)
Poland	861	MAT ELISA	14.3 15.4		(Sroka *et al.*, 2011)
Portugal	254	MAT, cutoff 1:20 dilution	9.8	Age of animals (higher prevalence in animals less than 3 months of age)	(Lopes *et al.*, 2013)

Country	Number tested	Tests performed	Seropositive (%)	Risk factors identified/Comments	Reference
Romania	3595 (domestic) 150 (wild boar)	IFAT, cutoff 1:32 dilution	Backyard 30.5 Sows 12.4 Fattening pigs 0.0 Wild boar 16.0		(Paştiu *et al.*, 2013)
Serbia	488	MAT, cutoff 1:25 dilution	9.2		(Klun *et al.*, 2011)
Slovakia	970	ELISA	All 2.2 Sows 4.3 Slaughter pigs 2.1		(Turčeková *et al.*, 2013)
Spain	2970	MAT, cutoff 1:25 dilution	16.6	Age Sows compared to fattening pigs Lack of rodent control Presence of cats	(García-Bocanegra *et al.*, 2010b)
Spain (Catalonia)	1202	MAT, cutoff 1:25 dilution	19.0	Presence of cats Percentage mortality at weaning Presence of outdoor facilities for sows on farm	(García-Bocanegra *et al.*, 2010a)
Switzerland	270 (Domestic) 150 (Wild boar)	ELISA (meat juice)	23.3 6.7		(Berger-Schoch *et al.*, 2011a)
Taiwan	395	LAT, cutoff 1:32 dilution	10.1		(Tsai *et al.*, 2007)
USA (organic production)	33	MAT, cutoff 1:25 dilution ELISA	90.9		(Dubey *et al.*, 2012a)
USA	6238	ELISA	2.6 (21.6% herd prevalence)	Rodent control measures Carcass disposal methods	(Hill *et al.*, 2010)
Vietnam	587	MAT, cutoff 1:25 dilution	27.2		(Huong and Dubey, 2007)
West Indies (Grenada and Carriacou)	247	MAT, cutoff 1:25 dilution	23.1		(Chikweto *et al.*, 2011)
Cervids					
Finland - - -	 1215 135 17	Direct agglutination test, cutoff 1:40 dilution	 Moose 9.6 White-tailed deer 26.7 Roe deer 17.6		(Jokelainen *et al.*, 2010)

Country	Number tested	Tests performed	Seropositive (%)	Risk factors identified/Comments	Reference
Ireland	348	LAT, cutoff 1:64 dilution	Deer 6.6		(Halová *et al.*, 2013)
New Caledonia	29	ELISA	Rusa deer (feral) 13.8		(Roqueplo *et al.*, 2011)
Spain (Galacia)	160	DAT, cutoff 1:64 dilution	Rose deer (feral) 13.7		(Panadero *et al.*, 2010)

NS = Not Stated; MAT = modified agglutination test; LAT = latex agglutination test; DAT = direct agglutination test; IFAT = indirect fluorescence antibody test; IHAT = indirect haemagglutination antibody test; PCR = polymerase chain reaction; ELISA = enzyme-linked immunosorbent assay

[1] Two different methods were used for establishing the cut-off point for the analytical method and, consequently, two estimates of seroprevalence were derived

9 APPENDIX 2: EVALUATION OF ADVERSE HEALTH EFFECTS

9.1 Dose Response

No human dose-response information for *T. gondii* infection is available. A recent quantitative microbial risk assessment (QMRA) used dose-response information from mice as a surrogate for human dose-response (Opsteegh *et al.*, 2011a). The QMRA related to ingestion of the bradyzoites form of *T. gondii* from tissue cysts. The dose-response relationship is likely to be dependent on the form of the organism ingested, as well as the dose.

9.2 *T. gondii* Infection Overseas

9.2.1 Seroprevalence

Seroprevalence studies for general and specific human populations are summarised in Table 7.

Where information on trends in seroprevalence are available, a decline in seroprevalence over time is generally reported (Bobic *et al.*, 2011; de la Luz Galvan-Ramirez *et al.*, 2012; Jones *et al.*, 2007; Jones *et al.*, 2014a). A meta-analysis of Mexican studies estimated the rate of the decline in seroprevalence to be approximately 0.1% per year over a period of 60 years (de la Luz Galvan-Ramirez *et al.*, 2012).

A worldwide review of seroprevalence concluded that the lowest seroprevalence of *T. gondii* infection was reported from China, United Kingdom and parts of Scandinavia (<10%) (Pappas *et al.*, 2009). The next lowest prevalence regions were North America, remaining parts of Scandinavia and parts of central Asia and south-east Asia (10-20%). The highest prevalence of seropositivity was seen in parts of South America, Africa and Indonesia (>60%). Prevalence in New Zealand (20-40%) was similar to Australia, Chile and some parts of Europe, Africa, the Middle East and India. No consideration was given to the analytical methods used to determine seroprevalence.

Table 7: Seropositivity rates overseas in humans

Countries	Year	Test method	Seropositivity (%)	Risk factors identified	References
Europe					
France	1995 2003 2010	NS	Women of childbearing age 54.3 43.8 36.7		(Nogareda *et al.*, 2014)
France	2004	IgG by ELISA	Rural population 46.9		(Gilot-Fromont *et al.*, 2009b)
Netherlands	2006-2007	IgG by ELISA	26.0	Participants ≥20 years of age: • Living in urban areas • Living in North-West of country • Low educational level • Consumption of raw pork • Keeping a cat • Not having occupational contact with clients or patients Participants <20 years of age: • Keeping sheep or cattle • Consumption of raw unwashed vegetables • Putting sand in mouth	(Hofhuis *et al.*, 2011)
Poland	NS	IFAT and ELISA	School children 41.0	Higher prevalence in girls Raw meat consumption (boys only) Seropositive girls had significantly higher developmental ages and poorer school performance	(Mizgajska-Wiktor *et al.*, 2013)
Portugal	2009-2010	IgG and IgM immunoassays	Women of childbearing age - IgG 23.9 - IgM 1.5	Consuming unwashed raw fruit and vegetables Consuming cured or smoked processed pork products Carrying out soil related activities without gloves	(Lopes *et al.*, 2012)
Romania (consolidated data)	NS-2009	Various	Pregnant women and women of childbearing age 3.7-75.4		(Dubey *et al.*, 2014b)
Serbia	2001-2005	NS	Women of childbearing age 33.0		(Bobić *et al.*, 2007)

Countries	Year	Test method	Seropositivity (%)	Risk factors identified	References
United Kingdom (London)	2006-2008	IgG by ELISA	Antenatal women 17.3	African/Afro-Caribbean, Middle Eastern or mixed ethnic origin Eating undercooked meat Consuming unpasteurised milk	(Flatt and Shetty, 2013)
Middle East					
Egypt (El Fayoum governorate)	2005-2006	IgG and IgM by ELISA PCR Sabin-Feldman dye test	Pregnant women (*n* = 59) - IgG 45.8 - IgM 30.5 - PCR 32.2 - Dye test 23.7 Non-pregnant women (*n* = 29) - IgG 41.4 - IgM 24.2 - PCR 27.6 - Dye test 17.2		(Ghoneim *et al.*, 2009)
Egypt	NS	IgG by ELISA	Blood donors 59.6	Consuming meat products Low educational status	(Elsheikha *et al.*, 2009)
Iran (Mazandaran province)	2006-2007	IgG by ELISA	School children 22.0	Presence of cats Contact with soil Eating raw or undercooked meat	(Sharif *et al.*, 2010)
Saudi Arabia	NS	Latex agglutination, indirect haemagglutination, IgG/IgM by ELISA	Females 28.2-41.9 (IgM 5.7) Males 20.0-38.0 (IgM 0.0) Infants 6.3-25.0 (IgM 0.0)		(Eisa *et al.*, 2013)
Saudi Arabia	NS	IgG and IgM by ELISA	Pregnant women - IgG 51.4 - IgM 8.8	Increasing age Rural residence Low family income Frequent consumption of undercooked meat Previous obstetric problems	(Al Mohammad *et al.*, 2010)
Turkey	NS	IgG/IgM by ELISA	Pregnant women - IgG 37.5 - IgM 0.0	Consumption of raw meat Working in agriculture	(Dogan *et al.*, 2012)
North America					

Countries	Year	Test method	Seropositivity (%)	Risk factors identified	References
Canada (northern Saskatchewan)	2011	IgG by ELISA	13.9	Age Non-ownership of pets	(Schurer *et al.*, 2013)
USA (Wisconsin state)	1997-1999	IgG by ELISA	Children 2-18 years 8.0	Living on a farm	(Munoz-Zanzi *et al.*, 2013)
USA	1999-2004	IgG by ELISA	6-11 years 3.6 12-19 years 5.8 20-29 years 10.1 30-39 years 14.3 40-49 years 15.7 Total 10.8	Increasing age Gender, greater prevalence in males Lower socioeconomic status	(Jones *et al.*, 2007)
USA	2009-2010	IgG by ELISA	6-11 years 1.5 12-19 years 4.1 20-29 years 9.3 30-39 years 10.5 40-49 years 14.8 50-59 years 15.0 60-69 years 17.4 70+ years 29.9 Total 13.2		(Jones *et al.*, 2014a)
Central America					
Mexico	2005-2006	IgG and IgM by ELISA	Psychiatric patients Controls - IgG 8.9 - IgM 2.2	Sexual promiscuity Unwashed raw fruit consumption History of surgery Protective: Lamb meat consumption	(Alvarado-Esquivel *et al.*, 2006)
Mexico	2006	IgG and IgM by ELISA	Healthy blood donors - IgG 7.4 - IgM 1.9	Presence of cats at home Increasing age Low educational status	(Alvarado-Esquivel *et al.*, 2007)
Mexico	2009-2010	IgG and IgM by ELISA	Workers occupationally exposed to raw meat - IgG 6.5 - IgM 4.0 Controls - IgG 8.9 - IgM 1.6	Two butchers who reported consuming dried meat were both seropositive	(Alvarado-Esquivel *et al.*, 2011d)

Countries	Year	Test method	Seropositivity (%)	Risk factors identified	References
Mexico	2011	IgG and IgM by ELISA	Drivers involved in automotive accidents - IgG 33.9 - IgM 1.9 Controls - IgG 36.0 - IgM 1.2	IgG antibody titres were significantly higher in drivers than controls No signs of ocular toxoplasmosis were found in the cohort	(Galván-Ramírez *et al.*, 2013)
Mexico	2009-2010	IgG and IgM by ELISA	Liver disease patients - IgG 13.3 - IgM 2.7 Controls - IgG 10.7 - IgM 3.3	For patients: Consumption of sheep, rabbit, venison and quail meat	(Alvarado-Esquivel *et al.*, 2011e)
Mexico	NS	IgG and IgM by ELISA	People with no contact with cats - IgG 25.0 - IgM 36.3	Consumption of pork Consumption of 'wildlife meat'	(Jimenez-Coello *et al.*, 2011)
Mexico (Durango state)	2010-2012	IgG and IgM by ELISA	Migrant workers - IgG 28,9 - IgM 20.8	Residence in rural area Consumption of unwashed raw vegetables Low frequency of eating away from home	(Alvarado-Esquivel *et al.*, 2013a)
Mexico (Rural Durango state)	NS	IgG and IgM by ELISA	General rural population - IgG 23.8 - IgM 2.2	Consumption of squirrel meat Consumption of turkey meat	(Alvarado-Esquivel *et al.*, 2008)
Mexico (Durango state)	2009-2011	IgG and IgM by ELISA	Workers involved with cultivation and selling of raw fruit and vegetables - IgG 7.5 - IgM 1.0 Controls - IgG 7.8 - IgM 2.8	Consumption of raw meat Consumption of unwashed raw fruit Living in a house with a soil floor	(Alvarado-Esquivel *et al.*, 2011a)

Countries	Year	Test method	Seropositivity (%)	Risk factors identified	References
Mexico (Durango state)	NS	IgG and IgM by ELISA	Mennonites - IgG 30.3 - IgM 3.3	Presence of cats Raising cattle Consumption of pigeon meat Consumption of untreated water	(Alvarado-Esquivel *et al.*, 2010b)
Mexico (Durango state)	NS	IgG by ELISA	Patients with: - Hearing impairment 8.2 - Haemodialysis 10.0 - Visual impairment 12.0 - Immunosuppression 6.8	Consumption of undercooked meat Consumption of raw cows' milk Raising animals Eating away from home	(Alvarado-Esquivel *et al.*, 2010a)
Mexico (Durango city)	NS	IgG and IgM by ELISA	General population - IgG 6.1 - IgM 2.1	Consumption of boar meat Consumption of squirrel meat Overseas travel (protective) Consumption of salami (protective)	(Alvarado-Esquivel *et al.*, 2011b)
Mexico (Durango city)	2009-2010	IgG and IgM by ELISA	People with recent work accidents - IgG 8.3 - IgM 0.8 Controls - IgG 5.3 - IgM 2.3	Boar meat consumption Negative association with: National trips Sausage consumption Ham consumption	(Alvarado-Esquivel *et al.*, 2012d)
Mexico	NS	IgG and IgM by ELISA	Aged subjects (≥60 years) - IgG 12.0 - IgM 2.9	Presence of cats in neighbourhood Consumption of boar, pigeon, iguana, armadillo and chorizo	(Alvarado-Esquivel *et al.*, 2012c)
Mexico	2013-2014	IgG and IgM by ELISA	Huicholes (ethnic sub-population - IgG 33.2 - IgM 22.0	Consumption of turkey meat	(Alvarado-Esquivel *et al.*, 2014a)
South America					
Brazil (consolidated data)	1974-2010	Various	Pregnant women 36.8-92.0		(Dubey *et al.*, 2012b)
Brazil	2011	IgG by ELISA	67.1	Increasing age	(Buery *et al.*, 2014)
Brazil (Santa Catarina)	2006-2007	IgG and IgM by ELISA	Solid organ donors - IgG 68.0 - IgM 0.0		(Do Amaral *et al.*, 2008)

Countries	Year	Test method	Seropositivity (%)	Risk factors identified	References
Brazil (Rio Grande do Sul state)	2002-2005	IgG by ELISA	Pregnant women - HIV positive 72.0 - HIV negative 67.1	The only infant with congenital toxoplasmosis was born to a women acute toxoplasmosis acquired during pregnancy. The woman did not have a high IgG result and was negative for IgM.	(Lago *et al.*, 2009)
Brazil (São Paolo state)	2009-2010	IgG by ELISA	Eye clinic patients 74.5	Presence of cats or dogs Consumption of raw or undercooked meat	(Ferreira *et al.*, 2014)
Brazil (Paraná state)	2007-2010	IgG and IgM by chemiluminescence	Pregnant women - IgG 51.7 - IgM 1.3	Residence in rural area More than one pregnancy Lower level of schooling Low income Raw or poorly cooked meat consumption Contact with soil	(Lopes-Mori *et al.*, 2013)
Brazil (Paraná state)	NS	IFAT	School children 46.4	Domestic cats in household Any kind of visual impairment	(Lopes *et al.*, 2008)
Brazil (Mato Grosso state)	NS	IFAT, cutoff 1:40 dilution	97.4		(Santos *et al.*, 2009)
Brazil (Salvador)	NS	IgG by ELISA	Children (4-11 years) 17.5	Greater number of siblings Cat at home House with non-treated piped water Absence of flush toilet at home	(Dattoli *et al.*, 2011)
Brazil (Amazonia)	2004	IgG by ELISA	Rural population (5-90 years) 65.8		(Ferreira *et al.*, 2009)
Chile	NS	IgG by ELISA	Pregnant women 39.1	Age of women	(Munoz-Zanzi *et al.*, 2010)
Colombia	NS	IgG by ELISA	Soldiers - Jungle operating 80.4 - Urban-based 45.1	Chorioretinal lesions were found in 4 soldiers who operated in the jungle and in 1 urban-based soldier	(Enrique Gomez-Marin *et al.*, 2012)
Asia					
Bangladesh (Mymensingh district)	NS	LAT, cutoff 1:32 dilution	0.0 (15 volunteers tested)		(Shahiduzzaman *et al.*, 2011)
China	2013	IgG by ELISA	All 21.6 Bai ethnic group 32.3 Han ethnic group 11.6	Consumption of raw pork and/or liver Cat feeding (Han only)	(Li *et al.*, 2014)

Countries	Year	Test method	Seropositivity (%)	Risk factors identified	References
China (Changchun)	NS	IgG and IgM by ELISA	Pregnant women - IgG 10.6 - IgM 0.0	Consuming raw or undercooked meat Consuming unwashed raw vegetables or fruit Contact with cats Living in a rural area Low educational status	(Liu *et al.*, 2009)
Japan	1997-2004	LAT, cutoff 1:32 dilution	Pregnant women, aged 16-46 years 10.3	Raw meat consumption	(Sakikawa *et al.*, 2012)
Kyrgyzstan	NS	IgG by ELISA	All 12.1 Urban 19.0 Rural 6.2	For rural cohort only: Age Poor living standard Number of sheep owned	(Minbaeva *et al.*, 2013)
Malaysia	NS	IgG and IgM by ELISA	Schizophrenia cases - IgG 51.0 - IgM 1.1 Controls - IgG 30.7 - IgM 1.1	Beef consumption Pork consumption Cat contact	(Juanah *et al.*, 2013)
South Korea	2010-2011	IgG by ELISA	17.0 (M 20.6, F 13.1)		(Ahn *et al.*, 2012)
South Korea	NS	IgG by ELISA	Rural pregnant women - Indigenous 40.6 - Immigrant 18.2	Consumption of raw/undercooked meat (particularly pork) Contact with cats Contact with soil	(Lin *et al.*, 2008)
Taiwan	2010	IgG and IgM by ELISA	IgG 9.3 IgM 0.3	Consumption of undercooked pork meat Raw mussel consumption Having a cat in the household Lower education level Donated blood in Eastern Taiwan	(Chiang *et al.*, 2012)
Africa					
Algeria	2006-2009	IgG and IgM by ELISA	Pregnant women - IgG 47.8 - IgM 1.1	Consumption of poorly cooked meat Exposure to cats	(Messerer *et al.*, 2014)

Countries	Year	Test method	Seropositivity (%)	Risk factors identified	References
Ethiopia	NS	IgG and IgM by ELISA	HIV seropositive cases - IgG 87.4 - IgM 10.7 Controls (healthy blood donors) - IgG 70.3 - IgM 3.0	HIV cases: Consumption of undercooked or raw meat Contact with cats Controls: Gender (higher prevalence in males) Consumption of undercooked or raw meat	(Walle *et al.*, 2013)
Ghana	NS	IgG and IgM by ELISA	IgG 84.4 IgM 6.4	Own cat Dispose of cat litter Exposed to soil Cats around the house Consumption of non-bottled water	(Abu *et al.*, 2014)
Mozambique	2010	LAT	HIV/AIDS patients 46.0	Consuming cattle meat Breeding cats or dogs Having regular contact with soil	(Domingos *et al.*, 2013)
Mozambique	NS	IgG by ELISA	Pregnant women 18.7 - HIV positive 31.3 - HIV negative 10.9		(Sitoe *et al.*, 2010)
Nigeria (Lagos)	NS	IgG by ELISA	HIV positive (*n* = 380) 54.2 Control (*n* = 80) 30.0	Lower educational ststus Consumption of beef	(Akanmu *et al.*, 2010)
Nigeria	NS	IgG and IgM by ELISA	Psychotic disorder cases - IgG 30.7 - IgM 7.1 Controls - IgG 17.9 - IgM 8.6	Age	(James *et al.*, 2013)

NS = not stated

An analytical method has been developed that targets antibodies to a specific 11 kDa sporozoites protein (Hill *et al.*, 2011). This enables an assessment of whether an infection has occurred through ingestion of oocysts (sporozoites-producing), rather than tissue cysts (bradyzoites-producing). Serum from acutely infected mothers (*n* = 76) who transmitted *T. gondii* to their foetuses *in utero* were examined (Boyer *et al.*, 2011). Sporozoites-specific antibodies, indicating that infection was associated with ingestion of oocysts, were detected in 78% of the cohort. Of the sporozoites-positive cases, approximately half identified a significant cat-associated risk factor. The results of this study also suggest that 22% of mothers may have acquired the infection through non-oocyst transmission route, such as ingestion of tissue cysts.

The same oocyst-specific antibody test was used to examine blood samples from pregnant women from Chile, who tested positive for exposure to *T. gondii* (IgG) (Munoz-Zanzi *et al.*, 2012). Oocyst-specific antibodies decrease with time. Analysis for these antibodies was restricted to blood samples that exhibited low IgG avidity (indicative of recent infection). Of the blood samples from women with evidence of recent *T. gondii* infections, 43.1% contained oocyst-specific antibodies. The authors of this study interpreted these results as indicated that up to 57% of cases may have been infected through meat tissue cysts.

9.2.2 Toxoplasmosis surveillance

9.2.2.1 Australia

The incidence of congenital toxoplasmosis has been estimated in a small study from south eastern Australia as 0.17 cases per 10,000 live births (Jayamaha *et al.*, 2012).

9.2.2.2 Brazil

Between 1998 and 2005, 41,112 pregnant women were assessed at a maternity health facility (Varella *et al.*, 2009). Acute toxoplasmosis was detected in 4.8 per 1000 pregnancies. Of 40,727 live births, 25 were considered to have congenital toxoplasmosis at birth (0.6 per 1000 live births). Twelve further congenital toxoplasmosis cases were diagnosed on follow-up, giving a total prevalence of 0.9 per 1000 live births. This equated to a transmission rate from mothers with acute toxoplasmosis of 18.5%.

A review of Brazilian studies on the prevalence of congenital toxoplasmosis found estimates in the range 0.5-2.3 cases per 1000 live births (Dubey *et al.*, 2012b).

9.2.2.3 England and Wales

Between 2008 and 2012, enhanced surveillance for toxoplasmosis was carried out in England and Wales (Halsby *et al.*, 2014). Laboratory-confirmed cases (*n* = 1824) were reported to the enhanced surveillance scheme, with the number of cases per year decreasing significantly, from 405 in 2008 to 311 in 2012, across the surveillance period. The majority of cases (*n* = 1109) were immunocompetent and non-pregnant. Most cases in this category presented with lymphadenopathy (*n* = 824), systemic (*n* = 168) or ocular (*n* = 163) symptoms.

There were 364 immunosuppressed, non-pregnant cases, of which 273 were HIV positive, 53 were transplant recipients and 38 had other causes of immunosuppression. When symptoms were reported, the most common symptoms involved the central nervous system.

Of 190 pregnant cases, 148 were asymptomatic, while 28 suffered a foetal loss or stillbirth. The dataset also included 33 congenital cases, of which 29 mother-child pairs were included in the dataset.

The UK Advisory Committee on the Microbiological Safety of Food published a risk profile in 2012 on *T. gondii* in the food chain.[18] It was estimated that 350,000 people in the UK become infected with *T. gondii* each year, of which 10-20% would be symptomatic. The importance of foodborne transmission in terms of burden of disease was inferred from the studies in the USA and Netherlands. The limited data on the prevalence and concentration of *T. gondii* contamination in meat and other foods was noted, along with the effect of control measures such as salad washing, milk fermentation, and curing.

9.2.2.4 France

A catalytic model was used to estimate the incidence, seroprevalence and seroconversion of *T. gondii* infection amongst women of childbearing age, from 1980 and extrapolating to 2020 (Nogareda *et al.*, 2014). For women 20 years of age, the incidence of *T. gondii* infection was estimated to decrease from 14/1000 susceptible population in 1980 to 3/1000 susceptible population in 2020. Similar decreases in incidence were estimate for other ages, with a 78% decrease in incidence from 1980 to 2020. The incidence of seroconversion in 2010 was estimated to be 2.5/1000 susceptible pregnant women.

9.2.2.5 Ireland

Mothers of congenital toxoplasmosis cases (n = 15) were interviewed to determine knowledge of and exposure to potential risk factors (Ferguson *et al.*, 2011). Seventy-three percent of mothers reported a lack of knowledge of risk factors for toxoplasmosis. Consumption of raw or undercooked meat during pregnancy was the predominant source of exposure to *T. gondii* cysts identified. Only one case reported contact with cats.

9.2.3 Sporadic Cases

Toxoplasmosis is usually asymptomatic or results in mild, self-limiting symptoms in immunocompetent adults.

A previously healthy 20 year old male presented with simple lymphadenopathy (swollen lymph nodes) (Taila *et al.*, 2011). The patient was otherwise asymptomatic. The symptoms did not resolve following a course of antibiotic treatment. After more detailed investigation, the patient indicated they had consumed a raw meat dish (beef or lamb) approximately one month previously. Blood tests revealed that the patient was seropositive for both IgG and IgM *T. gondii* antibodies.

A 16 year old girl was diagnosed with acute toxoplasmosis (Vitale *et al.*, 2014). The patient was suffering from fever, weakness, and body aches and presented with a swollen lymph node in the submandibular region. The patient was seropositive for both IgG and IgM *T. gondii* antibodies. The infection resolved within 8 months. The patient reported having tasted raw sausage, prepared from a backyard-raised pig. PCR analysis of the sausage showed evidence of *T. gondii* DNA.

[18] http://www.food.gov.uk/news-updates/news/2012/5298/toxoplasma accessed 22 April 2015

9.2.4 Outbreaks

Outbreaks of toxoplasmosis are rarely reported.

During December 2003 and January 2004, five adults, including two pregnant women, were hospitalised with multivisceral toxoplasmosis in a village in Suriname (South America) (Demar *et al.*, 2007). Amongst hospitalised cases, one fatality occurred as well as death of a foetus and a newborn associated with the two pregnant cases. A further three symptomatic cases and three asymptomatic cases were identified in the village, by serology and PCR. *T. gondii* was isolated from five cases and the isolates were found to be indistinguishable using genotypic techniques. While a number of risk factors for toxoplasmosis were present in the village environment, no source/cause of the outbreak was identified.

In 2010, 3 confirmed cases of toxoplasmosis in the same family were reported in France (Ginsbourger *et al.*, 2012). A meal including undercooked leg of lamb was implicated as the cause of the outbreak. *T. gondii* was isolated from the lamb meat.

A retrospective cohort study was conducted in the USA using medical records to examine instances where family members of acute toxoplasmosis cases were also tested for *T. gondii* infection (Contopoulos-Ioannidis *et al.*, 2013). Of 32 families identified, that met the study criteria, 18 (56%) included at least one other member who was found to have acute toxoplasmosis or recently acquired *T. gondii* infection. In all cases the serological characteristics of the additional family member were sufficiently similar to the index case to suggest a common source exposure. Commonly risk factors for family cases were identified for 9 of the 18 familial groups. Consumption of red meat was an identified risk factor in 7 instances. This study suggests that familial outbreaks of toxoplasmosis may be under-recognised.

9.2.5 Case-control studies investigating red meat and meat product consumption as a risk factor

A case-control study (148 cases, 413 controls) of toxoplasmosis cases, carried out in the USA, found significant adjusted odds ratios for (Jones *et al.*, 2009):

- Eating raw ground beef
- Eating rare lamb
- Eating locally-produced cured, dried or smoked meat
- Working with meat
- Drinking unpasteurised goats' milk, and
- Having three or more kittens

A separate model also found a significant risk associated with eating shellfish (Jones *et al.*, 2009). The highest attributable risks were associated with eating locally produced cured, dried or smoked meat (22%) and eating rare lamb (20%). Eating raw ground beef had an attributable risk of 7%.

A case-control study (175 cases, 278 controls) of mothers of children with congenital toxoplasmosis, carried out in Minas Geiras state, Brazil, found significantly elevated odds ratios for (Carellos *et al.*, 2014):

- Cats in the neighbourhood

- Owning or visiting homes with domestic cats
- Handling soil, and
- Eating fresh meat not previously frozen

Homes with a flush toilet and access to a treated water supply were found to be protective factors.

Case-control methodology was used to examine a cluster of acute toxoplasmosis cases in São Paolo state, Brazil (Jaguaribe Ekman *et al.*, 2012). After exclusions, 11 cases and 20 controls were interviewed. Consumption of green vegetables, but not meat or water, was significantly associated with acute toxoplasmosis.

A case-control study (30 cases, 224 controls) of laboratory-confirmed acute toxoplasmosis cases, carried out in Taiwan, found significantly elevated odds ratios for (Chiang *et al.*, 2014):

- Raw clam consumption
- Having a cat in the household
- Having HIV infection

Under-cooked meat (beef, pork and lamb) consumption was not a significant risk factor in this study, although the number of cases and controls who had eaten undercooked lamb and pork was small.

A case-control study of women of childbearing age (53 cases) in Serbia compared seropositive women to seronegative controls (Bobić *et al.*, 2007). Significantly elevated risk ratios were identified for:

- Consumption of undercooked meat, and
- Exposure to soil

When undercooked meat type was further examined, only consumption to beef had a significant elevated risk ratio.

9.3 Risk Assessment and Other Activities Overseas

9.3.1 Risk assessments considering *T. gondii* in red meat or red meat products

An Australian study conducted a qualitative risk assessment of the survival of *T. gondii* cysts in ready-to-eat (RTE) processed meat products (Mie *et al.*, 2008). Raw meat types were categorised according to the likelihood of containing *T. gondii* cysts. Literature information on the effectiveness of various processing steps for *T. gondii* cyst inactivation was then considered. It was concluded that the processes that may be most effective in inactivating *T. gondii* cysts were freezing, heat treatment, cooking, and the interaction between salt concentration, maturation time and temperature.

QMRA was used to quantify the relative contributions of sheep, beef and pork products to human *T. gondii* infections in the Netherlands (Opsteegh *et al.*, 2011a). The model used predicted that approximately two-thirds of meat-related *T. gondii* infections are due to consumption of beef products, despite the model employing a low prevalence figure (2%) for *T. gondii* contamination of beef. Forty percent of all predicted infections were estimated to be due to consumption of meat products that were not heated prior to consumption. When mincing

of meat was included in the model the predicted number of infections increased by a factor of seven and the contribution of beef products to total infections increased to 85%. It should be noted that this risk assessment predicts approximately 20 times the number of new cases of congenital toxoplasmosis per year as estimated from the observed incidence of IgM antibodies in newborns in the Netherlands (Kortbeek *et al.*, 2009)

9.3.2 Risk ranking of public health hazards associated with meat

As part of a review of meat inspection systems in the European Union (EU), biological hazards occurring in small ruminants (sheep and goats) that may be transmitted to humans were identified and ranked (EFSA, 2013a). The ranking process considered the prevalence and severity of the human health effects, and the evidence supporting a role for meat from small ruminants as a risk factor for human disease. This process resulted in classification of *T. gondii* as high priority, along with pathogenic verocytotoxin-producing *Escherichia coli. Bacillus anthracis, Campylobacter* spp. and *Salmonella* spp. were classified as low priority.

An equivalent process for farmed game animals ranked *T. gondii* as high priority for deer and wild boar (EFSA, 2013c), while for bovine animals a priority could not be determined for *T. gondii*, due to a lack of information on the role of beef consumption as a risk factor for human toxoplasmosis (EFSA, 2013b).

9.4 Burden of Disease due to Toxoplasmosis

9.4.1 Worldwide/global

A systematic review of the scientific literature was carried out to determine the global burden of congenital toxoplasmosis (Torgerson and Mastroiacovo, 2013). The global annual incidence of congenital toxoplasmosis was estimated to be 190,100 cases (95th percentile credible interval 179,300-206,300), with approximately one-third of cases in Africa. The region with the highest rate (cases per 1000 live births) was Central America (3.4 cases per 1000 live births, 95th percentile credible interval 2.5-4.1), while the lowest estimated rates of congenital toxoplasmosis were in Western Europe, North America and 'Western Pacific region A' (Australia, Brunei, Japan, New Zealand, Singapore) (0.6 cases per 1000 live births). The global burden of congenital toxoplasmosis, expressed in disability-adjusted life years (DALYs) was 1.2 million (95th percentile credible interval 0.76-1.90 million DALYs).

9.4.2 Australia

It was estimated that 3750 cases of acute foodborne toxoplasmosis occur in Australia each year (Kirk *et al.*, 2014). An expert elicitation estimated that foodborne transmission accounted for 31% (90th percentile credible interval 4-74%) of total acute toxoplasmosis cases (Vally *et al.*, 2014).

9.4.3 Canada

The estimated incidence of seropositivity to *T. gondii* derived for the USA (Jones *et al.*, 2007; Scallan *et al.*, 2011) was used to estimate the number of domestically-acquired foodborne cases of toxoplasmosis in Canada (Thomas *et al.*, 2013). Assuming that 3% of toxoplasmosis cases are travel-related and 50% are due to foodborne transmission, it was estimated that 9132 (90th percentile credible interval 6953-11628) domestically-acquired foodborne cases of

toxoplasmosis would have occurred in Canada in 2006. *T. gondii* infections were the 11th highest contributor to the total case burden of domestically-acquired foodborne disease in Canada and the highest ranked parasitic disease.

Since the study above, an expert elicitation process to estimate attribution of foodborne transmission for enteric pathogens for Canada has been published (Butler *et al.*, 2015). The estimate for foodborne toxoplasmosis was 51.4% (90% credible interval 8.8 – 82.7%).

9.4.4 Greece

An estimate of the burden of foodborne disease in Greece included assessment of toxoplasmosis (Gkogka *et al.*, 2011). The annual incidence of toxoplasmosis was estimated to be 3.4 cases per million population, with a case fatality rate of 3.8%. It was estimated that 50% of toxoplasmosis cases were due to transmission through food. The burden of toxoplasmosis, expressed in terms of disability-adjusted life years (DALYs), was 23 DALYs per million population. This was the fifth largest contribution to the overall burden of foodborne disease, after brucellosis, ill-defined intestinal infections, echinococcosis and salmonellosis.

9.4.5 Nepal

Based on data from a systematic review, it was estimated that 1396 ((5th percentile credible interval 1058-1780) incident cases of congenital toxoplasmosis would occur in Nepal each year, with approximately half of the cases being symptomatic (Devleesschauwer *et al.*, 2014). The annual burden of disease due to congenital toxoplasmosis was estimated to be 9255 (95th percentile credible interval 6135-13,292) DALYs. This was less than the burden estimate for neurocysticercosis, but an order of magnitude greater than the burden due to cystic echinococcosis.

9.4.6 Netherlands

A study to estimate the disease burden of foodborne pathogens in the Netherlands concluded that disease due to *T. gondii* infection was the single greatest contributor to the overall burden, as measured by DALYs (Havelaar et al., 2012). The total DALYs estimate for *T. gondii* was 3620, with the majority of the DALYs due to sequelae to the acute infection (chorioretinitis, intracranial calcifications, hydrocephalus and central nervous system abnormalities). The burden of disease due to congenital toxoplasmosis was estimated to be about twice that of acquired toxoplasmosis. The incidence of congenital toxoplasmosis was estimated from analysis of 10,008 dried blood spot filter paper cards from babies born in 2006 in the Netherlands for *T. gondii*-specific IgM antibodies (Kortbeek *et al.*, 2009). The estimated incidence of congenital toxoplasmosis was 1.8 cases per 1000 live births, with an associated DALY burden of 2300 DALYs. The next greatest contributors to the burden of disease due to foodborne pathogens were *Campylobacter* spp. and rotavirus. Foodborne transmission was estimated to account for 55% of the total burden of disease due to *T. gondii* infection, with red meat and red meat products estimated to account for 72% of foodborne transmission.

An update of this study for the 2011 year gave a similar estimate of the DALY burden (3570 DALYs) and estimated that the annual cost of toxoplasmosis in the Netherlands was €54.9 million (Mangen *et al.*, 2015).

9.4.7 USA

The annual incidence of acquired toxoplasmosis was estimated from seroprevalence studies (Jones *et al.*, 2007). By applying a proportion of 15% of seropositive cases as symptomatic cases (Scallan *et al.*, 2011), it was estimated that 173,415 (90th percentile credible interval 134,593-218,866) new symptomatic cases occur each year, with 50% of cases due to transmission of the parasite from food. A hospitalisation rate of 2.6% was estimated, resulting in 8889 (90th percentile credible interval 5383-13,203) estimated hospitalisations per year. It was estimated that 656 (90th percentile credible interval 409-952) fatalities would occur due to *T. gondii* infections. *T. gondii* infection was estimated to be the greatest contributor to domestically acquired foodborne disease due to parasites, although the number of cases estimated to be due to parasitic infections was an order of magnitude less than the estimates of disease cases due to bacterial or viral infections.

Using the same base data (seroprevalence) it was estimated that 1,075,242 people are infected with *T. gondii* in the USA each year (Jones and Holland, 2010). It was estimated that ocular toxoplasmosis lesions would occur in 2% of these cases, giving an estimate for the number of cases with ocular lesions per annum of 21,505. Using data from a large waterborne toxoplasmosis outbreak in British Columbia (Bowie *et al.*, 1997), it was estimated that 0.2-0.7% of infections would result in symptomatic retinitis, giving an annual estimate of symptomatic ocular toxoplasmosis for the USA of 2150–7527 cases.

Analysis of records for 521,655 live births during a 15 year period in a region of Northern California identified 2 cases of congenital toxoplasmosis, equating to a rate 3.8 cases per million live births (95th percentile confidence interval 1.5-9.2) (Jones *et al.*, 2014b). No infant deaths due to congenital toxoplasmosis were recorded during the same period.

Deaths due to toxoplasmosis (underlying or associated cause of death) were reviewed for the period 2000-2010 (Cummings *et al.*, 2014). In total 789 toxoplasmosis-related death were identified, with 271 due to toxoplasmosis encephalitis, 112 caused by other organ involvement, 71 due to congenital toxoplasmosis, 22 due to pulmonary toxoplasmosis and less than five deaths due to toxoplasma oculopathy. The greatest number of deaths (n = 240) were in the age range 35-44 years. Relative rates of death due to toxoplasmosis were greater for Black or Hispanic race/ethnicity and less for Asian/Pacific Islander and Native American, compared to white/Caucasian. Deaths per year decreased from 127 in 2000 to 44 in 2010. HIV infection was the co-morbid condition most commonly listed on death certificates (n = 440, 56%), followed by heart disease (n = 171). It was estimated that the 789 deaths represented 26,186 years of productive life lost (YPLL). Total productivity losses due to toxoplasmosis mortality in the period 2000-2010 was estimated to be $814.5 million.

Encephalitis hospitalisations and deaths were reviewed for the period 2000-2010 (George *et al.*, 2014). Of 48,596 encephalitis hospitalisations, 1416 (2.9%) were toxoplasmosis-related. The toxoplasmosis-specific hospitalisation rate for encephalitis decreased at an average of 8.4% across the study period (2000 to 2010). Age-specific rates for toxoplasmosis-related encephalitis was greatest for the age range 30-49 years. Mortality occurred in 8.8% of toxoplasmosis-related encephalitis cases, compared to 5.6% for all encephalitis cases.

A burden of disease estimate in DALYs for the USA was published in 2015 (Scallan *et al.*, 2015). Drawing on previously published estimates of incidence, the health states included were:

- Toxoplasmosis congenital: neonatal death; central nervous system abnormalities; intracranial calcification; hydrocephalus; chorioretinitis, onset soon after birth; chorioretinitis, onset later in life
- Toxoplasmosis acquired: mild, not hospitalised and recovered; severe, hospitalised and recovered; death; chorioretinitis.

The proportion of toxoplasmosis cases attributed to a foodborne source was 50%. The total number of DALYs was estimated as 32, 700, which was second only to non-typhoidal *Salmonella* (32, 900 DALYs). Stillbirths were excluded from the study.

APPENDIX 3: CONTROL MEASURES IN OTHER COUNTRIES

9.5 Control of Infections in Animals

9.5.1 Prophylactic use of antibacterials

Sulfadimidine (4 doses of 33 mg/kg body weight, intramuscular, administered over 48 hours) administered to pregnant goats in the fourth month of gestation was used a preventative measure against *T. gondii*-associated abortion (Giadinis *et al.*, 2013). The measure was effective in decreasing the rate of abortion in two goat herds from 20% to 1.25% and from 11% to 0%. PCR analysis of brain smears was used to confirm *T. gondii* as the cause of abortions that did occur.

Lambs (4 weeks old) experimentally infected with *T. gondii* were treated with the coccidiostat toltrazuril at weekly doses of 20 or 40 mg/kg, from 15 to 90 days post infection (Kul *et al.*, 2013). On study day 90, treated and control lambs were necropsied and brains and 11 muscle groups were examined for presence of cysts. All control animals had cysts in at least one muscle group, while 44% of animals in each toltrazuril dose group did not contain cysts in any examined tissue. The number of cysts observed was also significantly greater in the control groups than either of the treatment groups.

9.5.2 Vaccination

Vaccines have been developed for *T. gondii* infection control in stock animals.

Toxovax® is a vaccine for *T. gondii* infection, based on the S48 strain of *T. gondii*.[19] The S48 strain is incomplete and is not able to form tissue cysts or oocysts (Katzer *et al.*, 2014)

A seroprevalence study in sheep in Scotland found no significant difference in *T. gondii* seropositivity between animals from farms that reported using a vaccine (Toxovax®) and those that reported not using the vaccine (Katzer *et al.*, 2011). Given that the vaccine contains a live attenuated form of the parasite and is intended to elicit a humoral response, it is not possible to draw any useful conclusions from these seropositivity results.

Lambs were vaccinated with the S48 *T. gondii* strain and then vaccinated and unvaccinated lambs were challenged with a live oral dose of a complete strain (Katzer *et al.*, 2014). While not stated in the study, the vaccine used was probably Toxovax® or, at least, the same *T. gondii* strain incorporated into Toxovax®. Parasite DNA was found significantly less frequently in the vaccinated group (0.0% in heart tissue and 5.9% in skeletal muscle) than in the control group (75.0% in heart tissue and 87.9% in skeletal muscle). Control animals exhibited more frequent lesions in muscles and the central nervous system than vaccinated animals.

9.5.3 Farm construction factors

Seroconversion (seronegative animals becoming seropositive) was examined in seronegative feedlot sheep kept on either raised slatted floors or floor pens in a study carried out in Yucatan, Mexico (Hernández-Cortazar *et al.*, 2014). In floor pens, animals were directly in contact with soil. At the end of the fattening period (120 days), 9.6% of animals housed on raised slatted

[19] http://www.msd-animal-health.co.uk/Products_Public/Toxovax/Datasheet.aspx Accessed 28 January 2015

floors had seroconverted, while 42.3% of animals in floor pens had seroconverted. The relative risk of an animal in a floor pen seroconverting was 5.7 (95th percentile confidence interval 2.5-13.7).

9.5.4 Pest management

The role of rodents in transmission of *T. gondii* to pigs was investigated in a study in the Netherlands (Kijlstra *et al.*, 2008). A 4-month rodent control programme was carried out on three farms in a 7-month seroprevalence study, with seroprevalence assessed by real-time PCR. The rodent control programme was associated with a decrease in overall seroprevalence in pigs across the three farms from 10.9% to 3.3%. Two months after cessation of the rodent control programme seroprevalence had increased to 5.3%. Rodent control measures included trapping and baiting.

9.5.5 Measures at the abattoir

An assessment of microbial hazards and abattoir hygiene and inspection procedures concluded that these measures were generally ineffective for control of *T. gondii* infections in beef and pig meat (Blagojevic and Antic, 2014). This is consistent with conclusions reached by EFSA; that physical inspection of carcasses is ineffective for detection of *T. gondii* and current testing methods are insufficiently sensitive and are time consuming (EFSA, 2011; 2013a). It was also noted that, as *T. gondii* contamination cannot be passed from carcass to carcass, there is no advantage in segregating contaminated from non-contaminated carcasses. It was concluded that the best means of controlling *T. gondii* contamination was through a physical process, such as freezing.

9.5.6 Integrated approaches

An EFSA review concluded that a food safety assurance process for meat from small ruminants, to control *T. gondii* contamination, required an integrated approach involving farm-level and abattoir-level controls (EFSA, 2013a). Recommended measures specific to *T. gondii* include:

- Vaccination of cats, to reduce environmental loading of *T. gondii*, and vaccination of small ruminants to reduce infection and meat contamination.
- Serological surveillance and monitoring, preferably at the slaughterhouse. Due the high prevalence of seropositive herds/flocks, it was suggested that identification of seronegative flocks would be more useful. For example, meat from herds/flocks raised exclusively indoors, with good biosecurity and testing seronegative could be targeted to at-risk population groups or higher risk food products.
- Use of physical processes to control *T. gondii* in meat. Freezing was considered to be the most practical option.

A parallel review addressing farmed game reached similar conclusions (EFSA, 2013c).