



## **Deliverable D-JRP- TOXOSOURCES-WP2.3**

**Report on regional variation  
of prevalence and risk  
factors of human *T. gondii*  
infection within Europe**  
**Workpackage 2 of  
JRP22-FBZ4.1-  
TOXOSOURCES**

Responsible Partners:  
RIVM, SSI



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# D-JRP-TOXOSOURCES-WP2.3

## REPORT ON REGIONAL VARIATION OF PREVALENCE AND RISK FACTORS OF HUMAN *T. GONDII* INFECTION WITHIN EUROPE

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### TOXOSOURCES – PROJECT

This is a public deliverable of One Health EJP Joint Research Project:

**JRP22-FBZ4.1-TOXOSOURCES – *Toxoplasma gondii* sources quantified**

(<https://onehealthjep.eu/jrp-toxosources/>);

Work Package: **JRP-TOXOSOURCES-WP2 Multicentre quantitative microbiological risk assessment for *T. gondii* infections;**

Task: **JRP-TOXOSOURCES- WP2-T5 Review of prevalence and risk factors for human *T. gondii* infection.**

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

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

Objectives of TOXOSOURCES WP2:

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



To help achieve the goals of TOXOSOURCES WP2, within the task WP2-T5, an extensive systematic literature review was performed to provide accurate estimates of *T. gondii* prevalence in humans in Europe and to establish risk factors of the infection. The task was performed successfully in collaboration by scientists from related fields from several partner institutes across Europe. The work included integrative aspects in terms of collaboration and harmonising the process, and cross-sectoral aspects in terms of collaboration within the consortium and with task WP2-T2. Capacity-building was also included, and both experienced and early-career scientists participated in the work.

Dissemination of the outcomes is ongoing in collaboration with TOXOSOURCES WP1 and following the FAIR principles. Data have been provided to WP2-T1 for validation of QMRA outputs (Milestone M-JRP-TOXOSOURCES-15). The work and its results are being prepared for peer-reviewed scientific publication and the manuscript will be submitted to an Open Access journal. This Deliverable reports on the work done and highlights the key achievements of the process.

## **TOXOPLASMA GONDII**

*Toxoplasma gondii*, a protozoan parasite, is a common zoonotic foodborne pathogen in Europe and globally. If acquired during pregnancy, *T. gondii* can cause abortion and serious congenital disease. Infections acquired postnatally can cause ocular disease, and in particular atypical strains, which are common in areas outside of Europe, have caused severe toxoplasmosis even in immunocompetent individuals. Chronic and latent *T. gondii* infections cannot be considered harmless either, and the latent infections can reactivate.

Despite the severity of *T. gondii* and toxoplasmosis as a disease in humans, many questions remain unanswered, e.g. concerning the prevalence of *T. gondii* human infection, including geographical differences, the most important sources and routes of infection, and key risk factors, including food consumption habits. This lack of information has hampered the development of effective intervention strategies against toxoplasmosis in humans.

## **PURPOSE**

The aim of the work was to identify and compile prevalence and risk factor data for *T. gondii* infection in humans in Europe. The review was building on the approaches and experiences from systematic reviews performed previously in a COST-Action (COST-FBP (FA-1408) European network Food Borne Parasites) and for animal hosts in WP2-T2.

## **MATERIALS AND METHODS**

A systematic literature review on *T. gondii* prevalence in humans and associated risk factors was performed. To collect all relevant articles for the purpose of this review, a complex search algorithm, consisting of several individual search strings with Emtree-terms and a combination of boolean operators, was designed with the help of a librarian at RIVM. The search was performed using Embase database and was based on a list of inclusion criteria established and carefully followed to assess the eligibility of publications: only original peer-



reviewed articles published since the year 2000 were considered, which reported seroprevalence of *T. gondii* and/or risk factors for *T. gondii* positivity in humans, within Europe.

In total, the systematic literature search retrieved *T. gondii* seroprevalence data for **26 countries in Europe**, including EU member states. Risk factors were reported in studies from 17 of these 26 countries. To allow evaluation of geographical differences, the countries were aggregated into five regions as follows: East (Czechia, Estonia, Hungary, Latvia, Lithuania, Poland, Romania, Slovakia, Ukraine), North (Denmark, Finland, Norway, Sweden), Southeast (Cyprus, Greece, Serbia), Southwest (Italy, Portugal, Spain) and West (Austria, Belgium, Germany, France, Ireland, Luxembourg, Monaco, the Netherlands, Switzerland, the United Kingdom).

The systematic review was conducted according to the predefined protocol, following Cochrane guidelines and European Food Safety Authority (EFSA) guidance, and will be reported in accordance with PRISMA guidelines. The study selection was performed independently by **17 scientists from 14 institutes** (ANSES, FLI, INSA, ISS, PIWET, RIVM, RKI, SSI, SVA, UCM, UoS, WBVR, BIOR, UASVMCN) that are located in 12 countries across Europe, using the free online tool Cadima. An advantage of this broad international group of reviewers was the possibility to also include the non-English papers.

The screening process was divided into two parts: title and abstract screening, and fulltext screening. In both parts, each publication was screened by two randomly chosen screeners from the group, firstly at the level of title and abstract and after a consensus on inclusion between the two screeners was reached, full text screening was performed on the remaining publications with the inclusion criteria kept unchanged. If no agreement was reached, a third screener had a look at the article after which a decision was made. Reviews, case reports and articles with populations that were not representative were excluded (e.g. investigation of *T. gondii* infection in populations with specific diseases that could be a consequence of that infection).

The screening was followed by data extraction. Data extraction was performed after a trial on a subset of a few articles to ensure harmonised and accurate collection of data. The following data were extracted: number of participants tested and the number of positives, testing method, sample type, sampling years, country and region, age and gender ratio, and population specifics (for example pregnant women, general population, specific profession). In case a publication contained relevant data on more than one (sub)group, the data were included on separate rows in the dataset. During the data extraction, it became apparent that sometimes crucial information for interpretation of the extracted data was missing. Therefore, some articles were excluded in this phase.

## RESULTS

**A total of 1822 publications were retrieved** using the defined search strategy. After automated duplicate removal, titles, abstracts and full texts were screened and 142 articles met the inclusion criteria. In Table 1 the reasons for exclusion of articles are given, also separated for the two stages: during the full text screening (first stage, 225 exclusions) and at data extraction (second stage, 47 exclusion). After the screening and quality



assessment, data was extracted from 95 articles of which **91 articles gave information on seroprevalence and 22 articles reported data on risk factors** (Fig. 1).

Of the 91 articles reporting seroprevalence data, 51 provided seroprevalence data for women in reproductive age and/or pregnant women, 36 articles presented data for the general population, nine had (also) seroprevalences for children (< 18 years), eight articles reported seroprevalences in specified occupational groups, and eight studies were done in patient groups. Overall seroprevalences reported in the articles varied between 9.1% and 87.6%. Thirty studies were done in Eastern Europe (13.3%-74.4%), 27 studies in Western Europe (9.1%-87.6%), 19 in Southwestern Europe (12.0%-56.5%), 13 in Southeastern Europe (17.0%-70.9%) and five in Northern Europe (9.3%-26.7%). Three articles described studies from two regions: two articles with a study in Eastern and in Western Europe and one article with data from Eastern and Northern Europe. In 88 of the 91 articles the number of tested participants (in total 460,900) and positive participants (in total 140,576) was given.

Table 1: Reasons for exclusion during full text screening and quality assessment

Criteria	Number of exclusions* (first stage, second stage)
Does the record contain data about <u><i>Toxoplasma gondii</i> or toxoplasmosis</u> ?	12 (12, 0)
Is the record a <u>peer-reviewed</u> publication, PhD or doctoral thesis?	11 (11, 0)
Are the data on <i>T. gondii</i> or toxoplasmosis <u>original data</u> ?	36 (36, 0)
Is the data on <i>T. gondii</i> or toxoplasmosis presented (also) <u>from 2000 or newer</u> ?	54 (45, 9)
Does the record present data on <i>T. gondii</i> or toxoplasmosis <u>from Europe</u> (overseas territories excluded)?	94 (94, 0)
Does the record present data on <u>seroprevalence and/or risk factors</u> of <i>T. gondii</i> seropositivity and/or (postnatally acquired) human <i>T. gondii</i> infection or toxoplasmosis?	142 (114, 28)
Fulltext not available	10 (10, 0)
Fulltext not assessible	6 (6, 0)
Fulltext duplicate	6 (0, 6)
<b>No primary data/summary statistics are presented</b>	15 (10, 5)
<b>Basic (background) information is lacking</b>	10 (0, 10)

\* Articles may have been excluded based on more than one criterion.

Twenty-two articles describing risk factors of *T. gondii* infection were identified. One study investigated risk factors of acute cases of toxoplasmosis, the other studies were based on specific antibody (IgG) response screening of participants. Ten studies included women in reproductive age and/or pregnant women, seven the general population, four occupational groups and one a specific group (young adults living in a rural area with intensive livestock production). Eight studies were done in Western Europe, six in Eastern Europe, five in Southwestern Europe and two each in Northern and Southeastern Europe; one article described results from a study with participants in Eastern and Northern Europe. Seventeen of the 22 articles reported statistical significant risk factors. In the studies with multivariable analyses of the data, consumption of meat, especially raw/undercooked or from pork origin were mentioned as risk factors several times (Table 2). Consumption of raw milk (products), raw vegetables/fruit, and water were all reported once. Exposure to cats was the most



commonly reported risk factor of the different contacts with animals. Other factors reported several times are contact with soil, living rural/in villages or in big cities, and having specific, high-risk, professions.

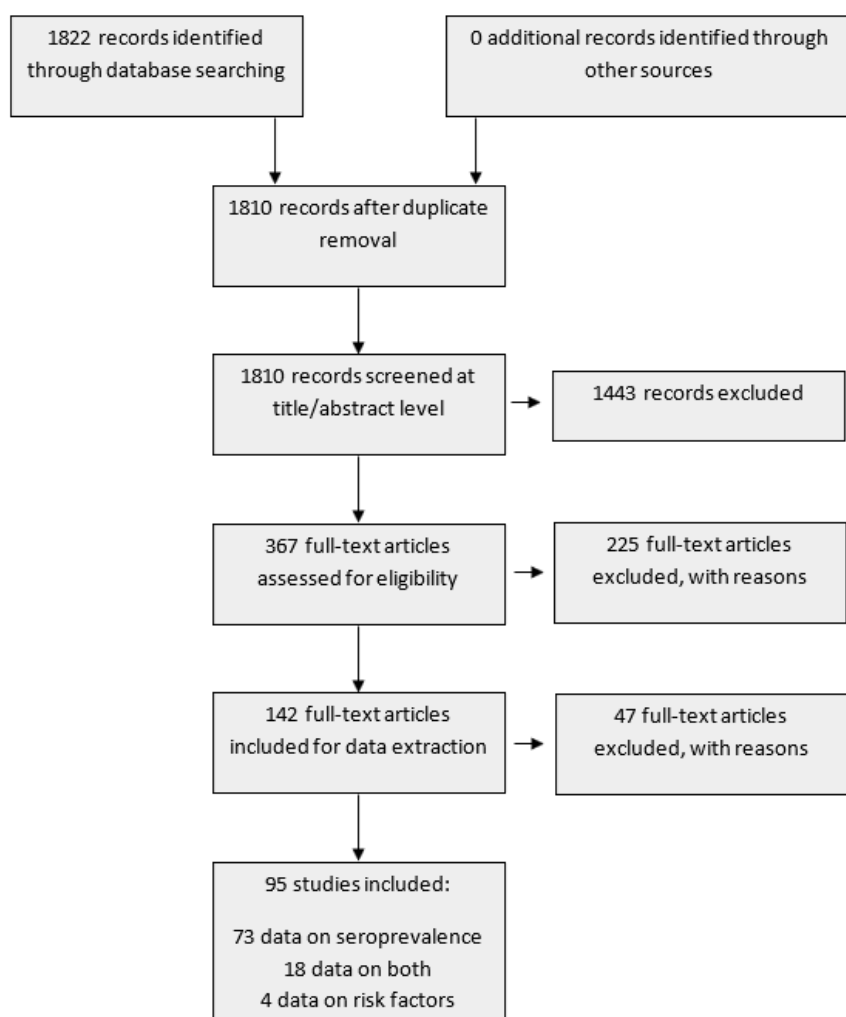


Figure 1. Screening process

Table 2. Statistical significant risk factors as mentioned in the studies in the review

risk factor	specification of risk factor	univariable	multivariable	unclear	Total*
<b>number of studies</b>		<b>11</b>	<b>12</b>	<b>1</b>	<b>17</b>
<b>consumption of meat</b>	consuming meat, sausages	3	1	1	4
<b>consumption of raw/undercooked meat</b>	consuming raw/undercooked meat	3	3	1	4
<b>consumption of beef</b>	consuming beef, fresh beef (steak, beefburger, tongue)	2			2
<b>consumption of lamb/mutton</b>	consuming lamb/mutton, fresh lamb chops	2			2
<b>consumption of pork</b>	consuming rare/undercooked/medium pork meat, processed	1	2		3



	pork products, fresh pork sausage				
<b>consumption of raw milk (products)</b>	consuming raw/unpasteurised milk, raw-milk cheese	3	1		3
<b>consumption of vegetables/fruit</b>	consuming raw vegetables/fruit	1	1		2
<b>consumption of water</b>	drinking all kinds of water including untreated sources (vs mineral water)		1		1
<b>farm contact</b>	living on a farm now, living on a farm during first 3 years of life	1			1
<b>exposure to animals</b>	regular contact with animal houses since the age of 0-3 years	1	1		1
<b>exposure to farm animals</b>	kept sheep or cattle in the past 5 years, contact with pigs	1			1
<b>exposure to domestic animals</b>	owned a rabbit, owned cat(s) and/or dog(s), had contact with domestic animals (not cats)	2	1	1	3
<b>exposure to cats</b>	owned or had contact with cat/kitten, ever/in the past 5 years/as child/as adult, often fed cat/kitten raw meat	2	3	1	5
<b>exposure to dogs</b>	owned a dog, often fed dog raw meat	2	1		2
<b>exposure to soil or garden</b>	exposure to soil (without gloves), contact with soil (vegetable) garden	3	2	1	5
<b>region of residence</b>	location of childhood home outside UK, living in the Netherlands in another region than southeast, living in north or central Portugal, living in east Germany (compared to west) or north/middle (compared to south)	2	3		4
<b>urbanization</b>	living in city/village with less than 50.000 inhabitants, at countryside/farm (now or as child), in urban areas, in cities with more than 100.000 inhabitants	4	5	1	7
<b>health status</b>	bloodgroup A/B/AB, having overweight/obesity, diabetes	2	2		2
<b>pregnancies</b>	more than 2 or 3 births, having had spontaneous abortions <sup>†</sup>	1	1		2



<b>marital status</b>	being married	1			1
<b>socio-economic status</b>	having a low socio-economic status	1			1
<b>educational level</b>	low educational level/primary school		2		2
<b>employment</b>	working with meat, having a small animal practice, high risk profession <sup>‡</sup>	2	1		3
<b>ethnic group</b>	African/Afro-Caribbean, Middle East, Mixed	1	1		1
<b>religion</b>	being Muslim	1			1

\* Some articles reported both univariable and multivariable results;

† Univariable: more than 3 births, spontaneous abortions; multivariable: more than 2 births

‡ High risk profession (as mentioned in the relevant article): i.e. agricultural industry, animal husbandry, veterinarians, butchers, slaughterers

## DISCUSSION AND PERSPECTIVES

The work was performed successfully as an international collaboration of scientists, also using the experiences and knowledge gained in TOXOSOURCES-WP2.2. The work and its results are of interest to the scientific community and will be disseminated via publication in an open access peer-reviewed international journal, planned for 2023, and presentations at scientific conferences or gatherings.

The *T. gondii* seroprevalence estimates will be analyzed by Bayesian hierarchical modeling and compared to the results from the multi-country QMRA. It is hypothesized that the countries with a high predicted incidence of *T. gondii* infection in the QMRA also have a higher reported seroprevalence. Moreover, the overview of risk factors will be compared against the most important sources of infection identified in the QMRA.