

Deliverable D-JRP-TOXOSOURCES-WP2.1

Report on prevalence of *T. gondii* infection in animals for human consumption and cats within Europe Workpackage 2 of JRP22-FBZ4.1-TOXOSOURCES

Responsible Partners: ANSES, INIAV, RIVM, SSI





GENERAL INFORMATION

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D-JRP-TOXOSOURCES-WP2.1 REPORT ON PREVALENCE OF *T. GONDII* INFECTION IN ANIMALS FOR HUMAN CONSUMPTION AND CATS WITHIN EUROPE

BACKGROUND

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

Work Package:

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

Task:

JRP-TOXOSOURCES- WP2-T2 Review of prevalence of T. gondii infection in animals.

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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-T2 an extensive systematic literature review was performed to provide accurate estimates of *T. gondii* prevalence in animals raised or hunted for human consumption, as well as in cats, which are definitive hosts for the parasite. 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-T5. 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 as input to WP2-T1 for the QMRA (Milestone M-JRP-TOCOSOURCES-10). The work and its results are being prepared for scientific article manuscript that will be submitted to an Open Access journal. The steps of the process were summarized into a conference abstract '*Toxoplasma gondii* prevalence in animals in Europe: a systematic review', which was selected for a poster presentation at the 3rd Annual Scientific Meeting of the One Health EJP in June 2021 in Copenhagen, Denmark; presenter: Filip Dámek.

This Deliverable reports on the work done and highlights the key achievements of the process.





PURPOSE

The aim of the work was to collate prevalence data for animals that are raised or hunted for human consumption as well as for cats in Europe. The review was building on the approaches and experiences from systematic reviews performed previously for European Food Safety Authority (EFSA) and in the Baltic and Nordic countries.

MATERIALS AND METHODS

For the first time in Europe, a systematic literature review on *T. gondii* prevalence was performed for **37 animal species**, ranging from domestic cats and livestock to wildlife. 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, based on a list of inclusion criteria established and carefully followed to assess the eligibility of publications. Only original peer-reviewed articles published in English since the year 2000 were considered, based on specified criteria including: natural infection, detection of *T. gondii*, geographical region, and defined host species (buffalo; cattle; felids – *Felis catus, Felis silvestris*, lynx; goat; equids – horse, donkey and mule; lagomorphs – rabbit and hare; pig; poultry – chicken / hen, duck, goose, turkey; sheep; wild birds – common teal, gadwall, eurasian pochard, eurasian wigeon, mallard, northern pintail, northern shoveller, pheasant, pigeon and wild duck; wild boar; wild ruminants – alpine chamois, deer, fallow deer, moose, mouflon, red deer, reindeer, roe deer, sika deer, Spanish ibex, white-tailed deer).

In total, the systematic literature search retrieved *T. gondii* prevalence data for **29 countries in Europe**, including EU member states. To allow evaluation of geographical differences between the prevalence in animal populations, 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 EFSA guidance, and will be reported in accordance with PRISMA guidelines. The study selection was performed independently by **20 scientists from 13 countries across Europe**, using the free online tool Cadima.

The screening process was divided into two parts: title and abstract screening, and full text screening. To harmonise the process across the screeners, a consistency check was performed before the screening process, using a set of determined criteria. First, the mentioned inclusion criteria were applied only to titles and abstracts. The studies that were considered eligible for full text screening were assessed by a smaller group of nine screeners to ensure consistency. The criteria were kept unchanged for both the title and abstract





screening, and the full text screening. Reviews, case reports and articles with populations that were not representative were excluded (e.g. investigation of *T. gondii* infection in an animal population with reproductive failure).

The screening was followed by data extraction. Data extraction was performed after a trial on five articles to ensure harmonised and accurate collection of data. The following data were extracted: number of animals tested and number of positive animals, testing method, sample type, sampling year(s), country and region, animal age, and outdoor access (for pigs, poultry, and cats). In case a publication contained relevant data on more than one animal population (a unique group of animals within the same age group) or more than one testing method was applied, the data were included on separate rows in the dataset. For data analysis, data based on direct detection methods (i.e. presence of the parasite demonstrated, e.g. by PCR) and data based on indirect detection methods (i.e. presence of antibodies to *T. gondii* demonstrated) were considered separately.

RESULTS

A total of 1985 publications were retrieved using the defined search strategy. After automated duplicate removal, titles, abstracts and full texts were screened and 275 articles met the inclusion criteria and were not excluded (Table 1). After quality assessment, **233 articles were eligible for data extraction** (Fig. 1).

Table 1. Reasons for exclusion during full text screening. Number of articles that were excluded in the full text screening step by the specific inclusion criteria that were not met.

Criteria	Number of exclusion(s)			
Defined species (Population)	16			
Toxoplasma gondii (Pathogen)	4			
Europe (Location)	23			
Data after the year 2000 (Year)	7			
Natural infection (Infection)	18			
Detection (Outcome)	10			
Relevance for the review (Relevance)	73			
Full-text availability (Full-text)	1			
Peer-reviewed (Reviewed)	4			
Original data (Data)	5			





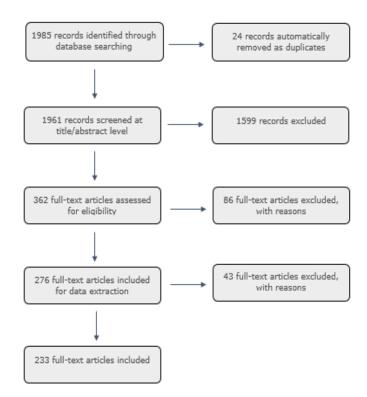


Figure 1. Screening process. Duplicate articles were removed before proceeding with title and abstract screening, full text screening, and data extraction. Non-eligible articles were excluded at each step.

The prevalence studies considered eligible for data extraction (Table 2) included a total of 55 articles on pigs involving 52317 animals, 48 articles on felids involving 116398 animals, 39 articles on sheep involving 39865 animals, 30 articles on wild boars involving 16326 animals, 29 articles on wild ruminants involving 15967 animals, 22 articles on goats involving 10754 individuals, 20 articles on cattle involving 21355 animals, 13 articles on lagomorphs involving 4822 animals, 12 articles on equids involving 1765 animals, 11 articles on poultry involving 8923 animals, 10 articles on wild birds involving 3273 animals, and one article on buffaloes involving 271 animals.

The number of different populations tested by direct / indirect tests were as follows: 19/85 for pigs, 30/81 for felids, 8/57 for sheep, 11/77 for wild boars, 11/109 for wild ruminants, 2/24 for goats, 12/46 for cattle, 3/22 for lagomorphs, 4/30 for equids, 0/31 for poultry, 3/16 for wild birds, and 1/3 for buffaloes (Table 2).





Table 2. Number of different populations / number of animals, by region and animal species.

Animal species	Test method	East	Mix	North	Southeast	Southwest	West	Total
Buffalo	Direct	1/74	0/0	0/0	0/0	0/0	0/0	1/74
	Indirect	3/197	0/0	0/0	0/0	0/0	0/0	3/197
Cattle	Direct	2/163	1/151	0/0	0/0	0/0	9/1096	12/1410
	Indirect	19/12631	1/234	0/0	0/0	5/962	21/6118	46/19945
Felids	Direct	3/71	0/0	4/467	0/0	8/1428	15/104321	30/106287
	Indirect	13/1755	0/0	9/2426	0/0	45/3943	14/1987	81/10111
Goat	Direct	2/89	0/0	0/0	0/0	0/0	0/0	2/89
	Indirect	7/1011	0/0	1/2188	7/2107	8/3695	1/1664	24/10665
Equids	Direct	1/82	0/0	0/0	3/105	0/0	0/0	4/187
	Indirect	8/802	0/0	0/0	6/1230	16/1765	0/0	30/3797
Lagomorphs	Direct	0/0	0/0	0/0	1/52	1/144	1/57	3/253
	Indirect	5/2430	0/0	2/215	1/105	11/865	3/954	22/4569
Pig	Direct	7/1404	0/0	0/0	1/44	6/1122	5/385	19/2955
	Indirect	18/10633	0/0	6/3695	2/791	36/17658	23/16585	85/49362
Poultry	Direct	0/0	0/0	0/0	0/0	0/0	0/0	0/0
	Indirect	10/1307	0/0	0/0	0/0	4/653	17/6963	31/8923
Sheep	Direct	2/107	0/0	0/0	1/1	0/0	5/730	8/838
	Indirect	8/4975	0/0	1/1940	5/2849	18/15057	25/14206	57/39027
Wild birds	Direct	2/630	0/0	0/0	0/0	1/5	0/0	3/635
	Indirect	0/0	0/0	0/0	1/72	14/2562	1/4	16/2638
Wild boars	Direct	4/509	0/0	0/0	0/0	6/703	1/150	11/1362
	Indirect	15/3477	0/0	10/1899	0/0	26/4869	26/4719	77/14964
Wild ruminants	Direct	0/0	0/0	0/0	0/0	7/237	4/124	11/361
	Indirect	13/2014	0/0	25/6153	0/0	34/4569	37/2870	109/15606
Total		143/44361	2/385	58/18983	28/7357	246/60237	208/162933	

Regarding the geographical distribution of different animal populations within the final set of articles, data from 143 populations comprising 44361 animals were collected from eastern region, from 58 populations comprising 18983 animals from northern region, from 28 populations comprising 7357 animals from southeastern region, from 246 populations comprising 60237 animals from southwestern region, and from 208 populations comprising 162 933 animals from western region (Table 2).

The proportion of positive animals out of all animals tested within a population varied by host species and region (Fig. 2).



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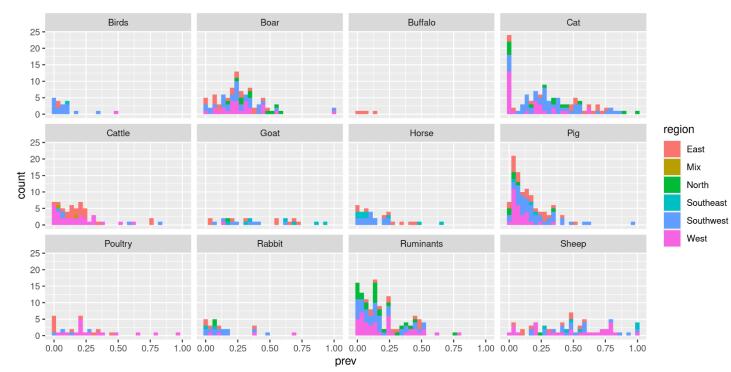


Figure 2. Histograms of the proportions of positive animals in a population, by animal species and region.

DISCUSSION AND PERSPECTIVES

The work was performed successfully as an international collaboration of scientists across Europe, and the complementary expertise in the multidisciplinary TOXOSOURCES consortium proved useful in the process. During the whole process, regular meetings were organized to discuss the work, and the experiences were shared with TOXOSOURCES-WP2-T5.

The work and its results are of interest to the scientific community and will be disseminated efficiently. The data, with emphasis on regional and age-related *T. gondii* prevalence estimates, will be analyzed by Bayesian hierarchical modeling and used as input data in the multi-country QMRA.

This work yielded a unique overview of *T. gondii* prevalence data in animals in Europe, and as input for the assessment of source attribution, these data can aid the development of effective prevention strategies.





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