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Review

Toxoplasma gondii exposure in wildlife in Spain: Is there any predictable threat for humans and domestic animals?

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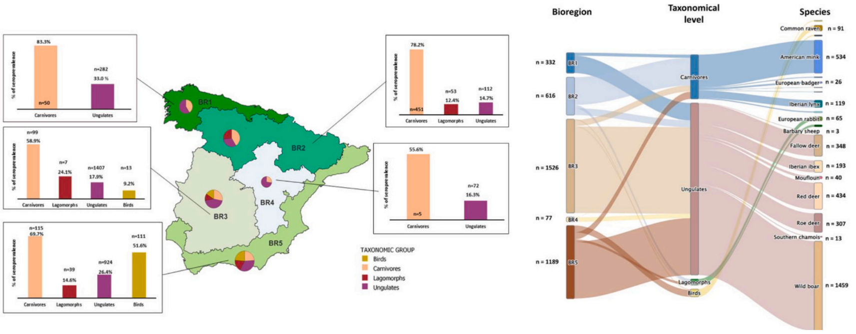
HIGHLIGHTS

- First review study of *Toxoplasma gondii* exposure in wildlife in Spain during 2000–2023
- Environmental variables, biodiversity and land use are related to *Toxoplasma gondii* exposure.
- There is a relationship between Toxoplasmosis human cases and environmental variables.
- The role of wildlife in the epidemiology of *Toxoplasma gondii* requires consideration.

GRAPHICAL ABSTRACT



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ABSTRACT

Toxoplasmosis is a parasitic zoonosis of key importance in veterinary and public health. This article summarizes the available data (from 2000 to 2023) of exposition to *Toxoplasma gondii* in wildlife species in Spain based on a systematic bibliographic search, as well as further analysis of its potential relationship with environmental variables, biodiversity, anthropogenic impact on the habitat, and the reported human cases of toxoplasmosis. The overall seroprevalence of *T. gondii* in carnivorous mammals, birds, ungulate and lagomorph species in Spain was estimated at 69.3 %, 36.4 %, 18.4 %, and 16.2 %, respectively. Among the studies considered, great

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Toxoplasmosis
Hospitalisation

heterogeneity was observed both between and within taxonomic groups [Cohen's $d > 0.8$; $X^2 = 1039.10$, $df = 4$ ($p < 0.01$) $I^2 = 97$ %, $r^2 = 1.88$, ($p < 0.001$)] and between and within bioregions [Cohen's $d > 0.5$; $X^2 = 368.59$, $df = 4$ ($p < 0.01$)]. The results of a generalized linear model explaining *T. gondii* seroprevalence in wild animals suggest the influence of abiotic variables [wetland ($p < 0.001$), unvegetated ($p < 0.001$), isothermality ($p < 0.001$), and mean temperature during wettest quarter ($p < 0.05$)] and number of intermediate host species as positively associated with increased exposure of wildlife to *T. gondii* ($p < 0.01$). *Toxoplasma gondii* DNA was detected in both wild birds and wild mammals (range: 0.0–51.2 %) mainly from north-centre, northeast, and central-west of Spain. Regarding hospitalisation rates due to toxoplasmosis in humans, some abiotic variables [permanent crops ($p < 0.05$) and mean temperature during wettest quarter ($p < 0.05$)] showed a positive association. Despite certain limitations, this research evidences a substantial gap of knowledge on the implication of wildlife in the life cycle of *T. gondii* in Spain. This lack of knowledge is particularly evident in areas where the human-livestock-wildlife interface overlaps, preventing us from accurately determining its true distribution in different habitats, as well as its potential direct or indirect implications on public and veterinary health.

1. Introduction

Toxoplasma gondii (phylum Apicomplexa) is an opportunistic obligate intracellular protozoan parasite that causes toxoplasmosis both in animals and humans (Aguirre et al., 2019). Toxoplasmosis is recognized as one of the most significant food-borne zoonoses worldwide, and it is estimated that approximately one third of the global population is infected with (or has been exposed to) *T. gondii* (Montoya and Liesenfeld, 2004; Calero-Bernal et al., 2023). In livestock, especially small ruminants, toxoplasmosis is mostly associated with reproductive failure resulting in significant economic losses (Dubey et al., 2020). *Toxoplasma* infection can be a serious threat to wildlife species that have not coevolved with this parasite (Innes, 1997; Mosquera et al., 2023). In immunocompetent humans, *T. gondii* infections are usually asymptomatic or may lead to a mild flu-like illness. In <10 % of infections, a mononucleosis-like syndrome with headache, malaise and fever occurs. Some patients may also present with fatigue, lymphadenopathy or ocular manifestations (Elsheikha et al., 2020). Reactivation of latent toxoplasmosis, in the absence of treatment, can cause fatal toxoplasmic encephalitis, myocarditis and/or pneumonitis in immunocompromised individuals (Smith et al., 2021). A primary infection in pregnant women could cause congenital transmission and consequent serious damage to the foetus (Montoya and Liesenfeld, 2004).

During the last few decades, most wildlife species (primarily wild ungulates) in Spain have spatially expanded due to different factors such as the intensification of game management practices, the human depopulation of rural areas, changes in land use, the introduction of individuals outside their native range, or the reintroductions of endangered species (Acevedo et al., 2011; Martínez-Abraín et al., 2020; Carpio et al., 2021). This situation has led to the overabundance of wild ruminant species in some areas, a phenomenon intensified by the increased demand for ecological and sustainable game meat in recent years (Carpio et al., 2021), also represent a potential source of environmental contamination by cysts/oocysts/eggs/spores of zoonotic parasites (Dashti et al., 2023) and, in addition, provide a reservoir for vector-borne pathogens (Tomassone et al., 2018). Thus, human infections by wildlife parasites have been identified and can be characterized as food-borne, vector-borne or environmentally transmitted.

Recently, the role of wildlife in the emergence and re-emergence of zoonotic diseases has gained increasing interest. In contrast to viral or bacterial zoonoses, zoonotic parasitic diseases from wildlife (such as toxoplasmosis) usually remain asymptomatic, have a limited interspecies spread and, due to their transmission routes and complex life cycles, do not cause outbreaks. These may be some of the reasons explaining why wildlife-associated zoonotic parasites receive less scientific and administrative attention within the context of the "One Health" approach (Polley, 2005).

To date, several surveys based on serological (detection of anti-*T. gondii* antibodies) and molecular (detection of *T. gondii*-specific DNA in tissues) approaches have been conducted to ascertain *T. gondii* exposure/infection rates in Spanish wildlife. In other countries of the Mediterranean basin such as France, Greece, Italy, and Portugal high seroprevalence rates have been detected in wild herbivores (range: 5.2–63.1 %) and carnivores (range: 0–100 %) (Beral et al., 2012; Coelho et al., 2014; Roqueplo et al., 2017; Waap et al., 2016; Rostami et al., 2017; Dini et al., 2023; Villa et al., 2023; Symeonidou et al., 2023). On the other hand, the presence in the environment of infected cats (domestic and feral) and wild felids (wildcat and Iberian lynx), all definitive hosts of *T. gondii*, implies potential environmental contamination with oocysts excreted with the faeces of infected animals. This, combined with favourable environmental and geographical factors (*e.g.*, temperature, precipitation, humidity, latitude) and anthropogenic activities (*e.g.*, land use), together with the consumption of game meat (hunting), can also maintain and increase the survival and transmission rate of this pathogen, and thus the risk of infection to animals and humans (Shapiro et al., 2019a; Zhu et al., 2023).

The main objectives of this research were i) to review and determine the occurrence of *T. gondii* infection in Spanish wildlife species, and ii) to evaluate the possible role of environmental and geographical variables and land use associated with an increased prevalence of toxoplasmosis cases in humans and wildlife. The results would help us to improve our current understanding of the role of wildlife and environmental factors in the life cycle of *T. gondii* and thus encourage public and veterinary health authorities and policy makers to design and implement effective measures to reduce animal and human infections within the framework of "One Health".

2. Materials and methods

2.1. Search strategy

To estimate the host range and spatial distribution of *T. gondii* infection in Spanish wildlife, a thorough systematic literature search was conducted using three peer-reviewed scientific databases including i) Scopus (Elsevier, The Netherlands), ii) PubMed (National Library of Medicine, USA), and iii) Web of Science (Thomson Reuters, USA). The search was carried out under strict accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Moher et al., 2009). To do so, we used a combination of the following search terms: "*Toxoplasma gondii*/toxoplasmosis" and "wild/wildlife" and "Spain". All the articles published between January 2000 until July 2023 either in English or Spanish, with at least an English abstract, were considered. Initial screening of manuscript titles and abstracts was performed independently by two researchers, which also assessed the full text of all potentially relevant studies and applied inclusion/exclusion criteria (see below).

2.2. Selection criteria

After extracting the records from the three databases, duplicate records were removed, and the titles and abstracts were screened for scientific relevance.

For article screening, the first step was to check the title and abstract to assess if the following eligibility criteria were fulfilled:

- Did the study target wildlife? Yes/No
- Did the studies were conducted in Spain? Yes/No
- Did the study include original results? Yes/No
- Did the study include seroprevalence/molecular data? Yes/No

Articles were only selected if answer to all questions was "Yes". Then, the following step was to carry out a complete reading of the full content of each article. The exclusion criteria were: i) publications on parasites other than *T. gondii*, ii) records documenting the detection of *T. gondii* in wild animals kept in captivity or domestic species, iii) studies that were conducted in countries other than Spain, and iv) publications including data out of the scope (Fig. S1).

2.3. Data extraction

After a comprehensive examination of selected articles, the following data were extracted and entered into Microsoft Excel worksheets (Tables S1 and S2): the author's last name, publication year, location, bioregion, and province (when it was possible to assign it, see below Section 2.5). We also recorded the common and scientific names of the investigated host species, sample size, the number of positive and negative cases, as well as information about the diagnostic tools used. In cases where multiple host populations were studied, the data were extracted separately for each host species.

Data on human toxoplasmosis cases were formally requested to the Specialized Health Care Registry (RAE-CMBD) of the Spanish Ministry of Health (Royal Decree 69/2015). Entries during the period 2000–2021 whose main or secondary diagnosis was "toxoplasmosis" were selected. We recorded the total number of hospitalizations due to toxoplasmosis per year and during the whole study period. The obtained data were stratified by province (Table S3).

2.4. Definitive and intermediate hosts

Data on wild host species distribution were obtained from the Spanish Inventory of Terrestrial Species website (BD_IEET, 2015). The dataset yielded 656,026 records; we extracted a total of 32,598 data for the following 21 species (19 intermediate hosts and 2 definitive/intermediate hosts): *Ammotragus lervia* (Barbary sheep), *Capra pyrenaica* (Iberian ibex), *Capreolus capreolus* (roe deer), *Cervus elaphus* (red deer), *Columba livia* (common pigeon), *Corvus corax* (common raven), *Dama dama* (fallow deer), *Felis silvestris* (European wildcat), *Herpestes ichneumon* (Egyptian mongoose), *Larus michahellis* (yellow-legged gull), *Lepus granatensis* (Iberian hare), *Lynx pardinus* (Iberian lynx), *Martes martes* (European pine marten), *Meles meles* (European badger), *Mustela putorius* (polecat), *Neovison vison* (American mink), *Oryctolagus cuniculus* (European rabbit), *Ovis aries* (mouflon), *Rupicapra pyrenaica* (southern chamois), *Sus scrofa* (wild boar), and *Vulpes vulpes* (red fox). Similarly, presence/absence data from domestic ruminants (cattle, pigs, goats, sheep) were extracted at the municipality level from the 2020 official census (INE, 2020). Using the 10 × 10 km UTM grid squares of mainland Spain as territorial unit, we obtained the sum of all host species present in each unit, separating the exposition (only serological data) to *T. gondii* records for intermediate and definitive host species. However, when rescaling the data to province level, and given that 100 % of the provinces showed the presence of the four livestock species, these species have not been taken into account in the statistical analysis.

2.5. Geographical distribution

Geographically, samples included in this review were collected throughout the five bioregions (BRs) of mainland Spain and on some Mediterranean islands (Fig. 1). These BRs were defined by the Spanish National Wildlife Disease Surveillance Plan based on the ecosystems, presence and abundance of wildlife, and climatological and epidemiological criteria of the wildlife communities present (Muñoz et al., 2010; PNVSFS, 2020). Briefly, BR1 has an Atlantic climate, with mild temperatures and high average annual rainfall. Bordering this BR to the

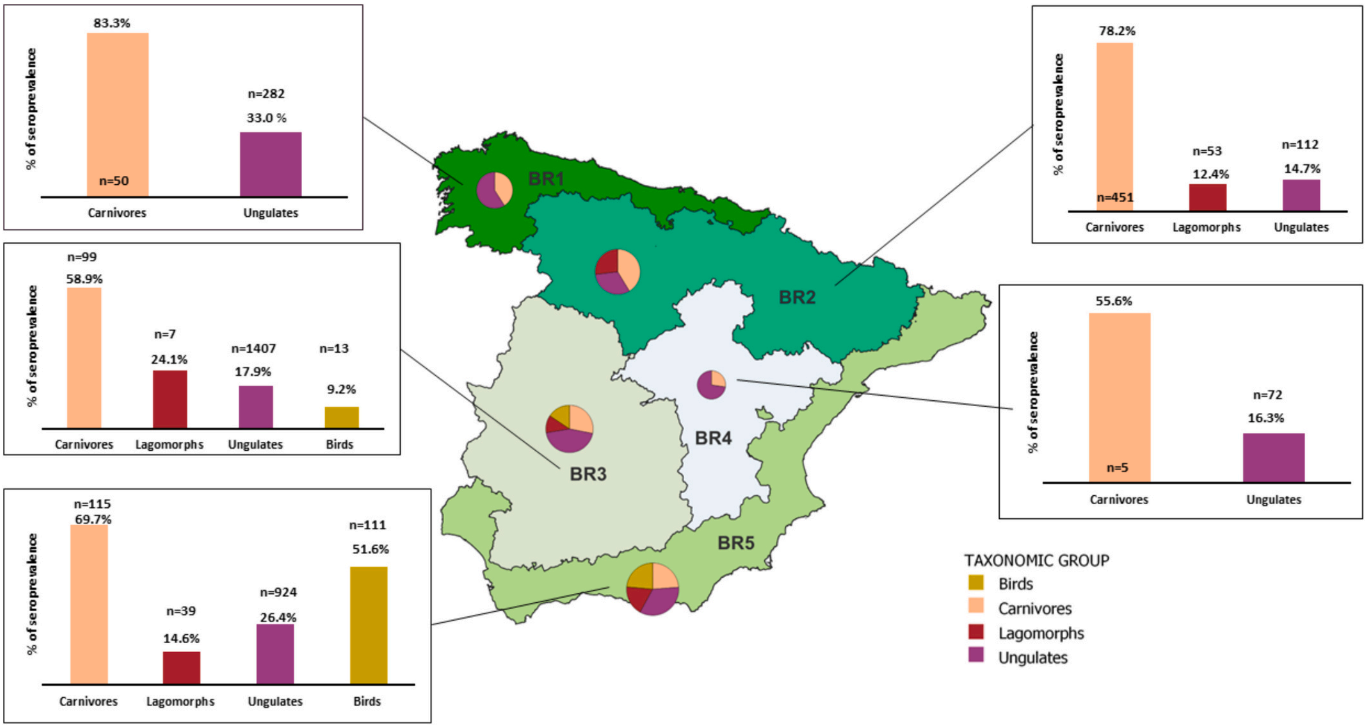

Fig. 1. Map showing the geographical distribution and seroprevalence of anti-*Toxoplasma gondii* antibodies in different taxonomic groups of wild animals for the five bioregions.

south lies BR2, with a continental Mediterranean climate and predominance of cereal crops. BR3 extend over the south-central part of the country, a region with low altitude mountains and cold winters, hot and dry summers, and rainy autumns and springs. The BR4 covers inland mountains with a continental Mediterranean climate. Finally, along the south and east coast extends BR5 with warm, rainy winters, and hot and dry summers.

2.6. Corine and WorldClim variables

The eco-geographical variables incorporated in the model included 19 bioclimatic variables and altitude data from the WorldClim website (Fick and Hijmans, 2017) at the 30 s (~1 km^2) spatial resolution. Land use data were compiled from the CORINE database (Corine Land Cover, 2018), which was provided by the European CORINE Land Cover project (Heymann et al., 1994) and then aggregated to obtain 13 different land use variables, based on their significance in explaining the distribution of fauna (Table 1). To transfer the above-mentioned information to our territorial units (10 × 10 km UTM grid squares), we used the zone statistics plugin of the software QGIS (version 3.28.6). Through this procedure, the medium value of every eco-geographical variable was calculated for each territorial unit. The data were rescaled to the province-level for the statistical analysis and to the bioregion-level for the descriptive analysis.

Table 1

List of variables used in the models.

Factor	Variable	Resolution
Abiotic	Bio 1: annual mean temperature[b]	1 × 1 km (raster)
	Bio 2: mean diurnal range[b]	
	Bio 3: isothermality[b]	
	Bio 4: temperature seasonality[b]	
	Bio 5: max temperature during warmest month[b]	
	Bio 6: min temperature during coldest month[b]	
	Bio 7[a]: annual temperature range[b]	
	Bio 8: mean temperature during wettest quarter[b]	
	Bio 9: mean temperature during driest quarter[b]	
	Bio 10: mean temperature during warmest quarter[b]	
	Bio 11: mean temperature during coldest quarter[b]	
	Bio 12[a]: annual precipitation[b]	
	Bio 13: precipitation during wettest month[b]	
	Bio 14: precipitation during driest month[b]	
	Bio 15: precipitation seasonality[b]	
	Bio 16: precipitation during wettest quarter[b]	
	Bio 17: precipitation during driest quarter[b]	
	Bio 18: precipitation during warmest quarter[b]	
	Bio 19: precipitation during coldest quarter[b]	
	Elevation[b]	1 × 1 km (raster)
	Wetland (4.1; 4.2)[c]	0.1 × 0.1 km
	Heterogeneous agricultural areas (2.4)[c]	
	Permanent crops (2.2)[c]	
	Artificial areas (1)[c]	
	Croplands[a] (2.1.1)[c]	
	Irrigated arable (2.1.2; 2.1.3)[c]	
	Agroforestry areas (2.4.4)[c]	
	Coniferous forest[a] (3.1.2)[c]	
	Broad-leaved forest (3.1.1)[c]	
	Mixed forest (3.1.3)[c]	
	Grassland pastures (2.3.1; 3.2.1)[c]	
	Shrub (3.2.2; 3.2.3; 3.2.4)[c]	
	Unvegetated (3.3)[c]	
Biotic	Number of intermediate host species per grid[d]	10 × 10 km
	Number of definitive host species per grid[a, d]	
	Toxoplasma gondii exposition in wild animals	1 × 1 km
	Human population density	

[a] Collinear variable excluded from the models, using the variance inflation factor, with the threshold cut-off value being set at 3.

[b] WorldClim.

[c] Corine.

[d] Spanish Inventory Terrestrial Species 2014 (BD_IEET, 2015) [23].

2.7. Statistical analysis

Prior to modelling, we proceeded to exclude collinear variables using the variance inflation factor (VIF), with the threshold cut-off value being set at 3 (Zuur et al., 2010). We designed two generalized linear models (GzLM), with a binomial distribution and logit link function, to explore the explanatory variables in relation to the response variable (seroprevalence of toxoplasmosis in wildlife and prevalence in humans). The first one was defined as the ratio between seropositivity to *T. gondii* and total animals, and the second, as the ratio between the mean value of hospitalized patients due to toxoplasmosis (calculated between 2000 and 2021) and the total number of inhabitants by province using official census data (INE, 2020). For the human toxoplasmosis analysis, the frequency of antibodies in wild animals was considered as an explanatory variable together with human density. The selection of the 'best model' was made using the corrected Akaike's information criterion (AICc). In our case, it was obtained carrying out a backward stepwise procedure. The statistical analysis was done using R v4.2.2 (R Core Team, 2020). The model's residuals were examined by Moran's I test for residual spatial autocorrelation to detect spatial structures (Diniz-Filho et al., 2003). The assumptions of binomial GzLMs were met in both models (Zuur et al., 2009).

To represent the data, we designed a map showing the bioregions with five different pie charts and bar graphs (Fig. 1). Only host species with at least 10 individuals seropositive to *T. gondii* were represented on the map. To display graphically the information about the total number of *Toxoplasma*-positive cases by bioregion, the data on the number of positive cases by species were logarithmically transformed for ease of visualization. For the bar charts we represented only the seroprevalence by selecting for each bioregion the five species with the highest prevalence rates and specifying for each one the corresponding number of positive individuals.

The effect size, Cohen's *d*, was calculated by taking the difference of the means of two groups and dividing it by the weighted pooled standard deviations of these groups. According to Cohen (1992), effect sizes of 0.2–0.3 were considered small, 0.5 as medium, and ≥0.8 as large. This enabled us to compare the effect size between pairs of bioregions and pairs of taxonomic groups. In the case of multispecies groups, it was possible to compute *Cohen's d* (Cohen, 1988) and consider the dispersion of group means. This allowed us to compare the effect size across all bioregions and taxonomic groups. Study bias and heterogeneity at the study level were calculated using inverse variance index (I^2), with I^2 values categorized as low, moderate, and high at 25 %, 50 %, and 75 %, respectively (Higgins et al., 2003).

We also designed a Sankey diagram (Fig. 2) to show the interrelational intensity among the three main categories considered: the bioregion of origin (BR1, BR2, BR3, BR4, BR5), the taxonomical level of the host (birds, carnivores, lagomorphs and ungulates) and the presence of host species where *T. gondii* exposure has been reported. The *n* represented the number of seropositive animals; the three categories were represented by nodes and connected by edges. We defined the frequency of association between nodes by the size of each edge. The packages used in R were 'highcharter' (Kunst et al., 2017) and 'htmlwidgets' (Vaidyanathan et al., 2021).

3. Results

3.1. Literature search and article selection

A total of 499 articles were obtained from the search process. Of them, 33 articles were shortlisted for data extraction after thorough revision and removal of duplicates (Fig. S1). Of them, 27 articles were based on the analysis of serum samples from wildlife species, whereas the remaining five articles used molecular (PCR) methods for the detection of the parasités DNA in tissue samples. The literature review showed that the presence *T. gondii* has been investigated in 96 host

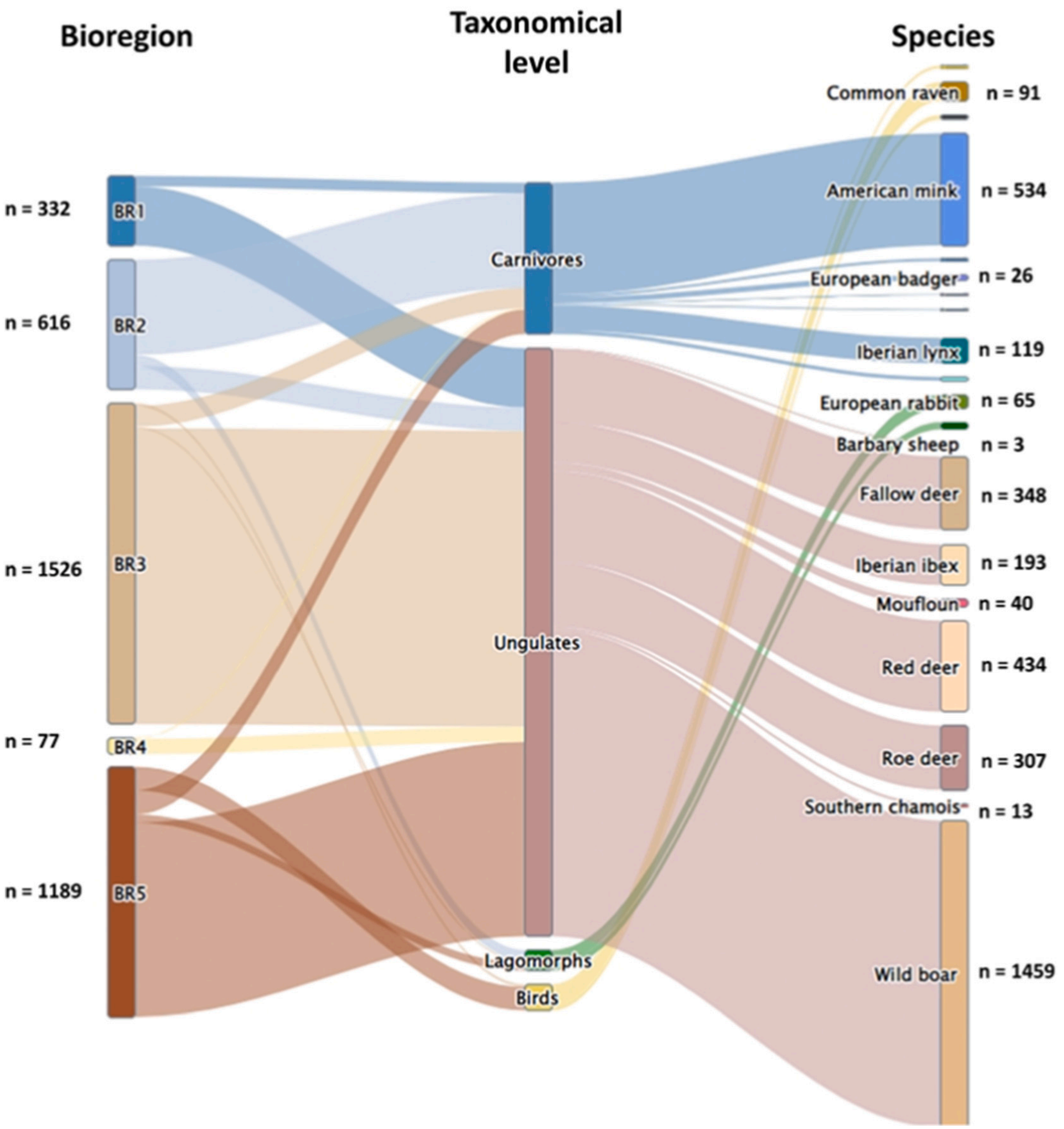

Fig. 2. Taxonomical level and wildlife host species exposed to *Toxoplasma gondii* by bioregion (BR) of origin. Host species (common pigeon, yellow-legged gull, Egyptian mongoose, European wildcat, red fox, Iberian hare, polecat, and pine marten) with *n* values <3 were not represented in the diagram.

species belonging to 34 families and 23 orders of free-roaming mammals and birds; subsequently, anti-*T. gondii* antibodies and/or DNA have been detected in 67.7 % (65/96) of the host species analysed (Table S1).

3.2. Prevalence of Toxoplasma gondii based in serology

Serological diagnostic techniques were used in 27 papers, in which serum samples of 17,256 individuals were analysed. Diagnostic methods used to evaluate circulating anti-*T. gondii* antibodies in wildlife included in-house modified and direct commercial agglutination test (MAT/DAT) (23 studies), commercial enzyme-linked immunosorbent assay (ELISA) (three studies), and in-house indirect fluorescent antibody test (IFAT) (one study). The pooled seroprevalence of *T. gondii* in Spanish carnivores, birds, lagomorphs, and ungulates was estimated at 71.3 % (920/1290; 95 % CI: 68.79–73.72), 27.3 % (498/1819; 95 % CI: 25.38–29.47), 13.7 % (99/724; 95 % CI: 11.36–16.37), and 21.2 % (2800/13,198; 95 % CI: 20.53–21.92), respectively. The best-represented host species was wild boar, where seroprevalence of anti-*T. gondii* antibodies was 23.8 % (1462/6132; CI 95 %: 22.79–24.92), followed by red deer (14.7 %, 434/2959; CI 95 %: 13.44–15.99), fallow deer (27.6 %; 348/1260; CI 95 %: 25.22–30.15), roe deer (26.6 %; 307/1155; CI 95 %: 24.11–29.20), and Iberian ibex (18.0 %; 193/1071; CI 95 %: 15.83–20.44). Seroprevalence rates of 100 % were observed in northern pintail (*Anas acuta*), European goldfinch (*Carduelis carduelis*), common coot (*Fulica atra*), common moorhen (*Gallinula chloropus*), Eurasian otter (*Lutra lutra*), pine marten, and polecat. However, sample sizes for those species were ≤ 6 individuals (Table S1). Subgroup analyses in terms of taxonomic groups showed a high heterogeneity ($I^2 > 75$ %, $p < 0.01$) among the studies considered, except in the taxonomic group of lagomorphs ($I^2 = 28$ %, $p = 0.25$) (Table 2).

3.3. Geographical distribution of Toxoplasma gondii based on serological data

Due to the strong effect size observed among taxonomic groups (Cohen's $d > 0.8$) and a moderate effect size for BRs (Cohen's $d > 0.5$) (Table S4), we were unable to combine and provide an average seroprevalence value per BR. Instead, we showed an average seroprevalence value for each taxonomic group in each bioregion (Fig. 1). On the other hand, heterogeneity between BRs was high ($I^2 > 84$ %, $p < 0.01$) and is shown in Table 2. Seroprevalence rates in Spanish wildlife according to their taxonomic group and BR of origin was 83.3 % (50/60) in carnivores and 33.0 % (282/855) in ungulates in BR1, 78.2 % (451/577) in carnivores, 12.4 % (53/428) in lagomorphs and 14.7 % (112/761) in ungulates in BR2; 58.9 % (99/168) in carnivores, 24.1 % (7/29) in lagomorphs, 17.9 % (1407/7850) in ungulates, and 9.2 % (13/142) in birds belonging to BR3; 55.6 % (5/9) in carnivores and 16.3 % (72/442) in ungulates belonging to BR4; and 69.7 % (115/165). In carnivores, 14.6 % (39/267) in lagomorphs, 26.4 % (924/3495) in ungulates, and 51.6 % (111/215) in birds in BR5 (Fig. S2). This result was similar to that for BR5 ($n = 1189$), which ranked second in terms of *Toxoplasma*-positive wild animals (Fig. 2). The wild boar was again the species with the largest sample size ($n = 529$), followed by red deer ($n = 183$), fallow deer ($n = 162$), and common raven ($n = 91$). The common raven also stands out for its prevalence of infection, reaching 80.5 % (91/113), second only to the American mink (82.6 %, 38/46) (Fig. 1). High or very high *Toxoplasma*-positivity rates were reported in several host species in BR1 including the pine marten (100 %, 4/4), the polecat (100 %, 1/1), the American mink (81.8 %, 45/55), the fallow deer (51.7 %, 62/120), and the red deer (35.9 %, 37/103). In contrast, BR4 harboured the lowest number of *Toxoplasma*-positive animals (1.2 %; 80/460) (Fig. 2), with three ungulate species (fallow deer: 27.9 %, 24/86; roe deer: 20.0 %, 18/90; and red deer: 13.7 %, 17/124) contributing similarly to the burden of the disease in this bioregion.

3.4. Occurrence of *Toxoplasma gondii* based on PCR

A total of 1144 tissue (brains and/or hearts tissues) samples of 30 different host species were analysed by molecular methods in six studies (Table S2), parasite DNA was found in 7.9 % (90/1144) of the species studied. *Toxoplasma gondii* DNA has been detected in both wild birds and wild mammals mainly from north-centre, northeast and central-west of Spain (Table S2).

3.5. Geographical distribution of *Toxoplasma gondii* based on molecular data

Toxoplasma gondii DNA detection in Spanish wildlife has been investigated in both wild birds and wild mammals mainly from north-centre (Burgos and Madrid), central-west (Cáceres and Badajoz) and northeast (Catalonia) of Spain, all areas belonging to bioregions BR2-BR5. The highest number of *Toxoplasma*-positive animals was detected in carnivores and ungulates from BR3 with the red fox (51.2 %; 21/41), fallow deer (47.6 %; 10/21), and Iberian ibex (46.2 %; 12/26) as the most affected species (Table S2). Regarding the detection of *T. gondii* DNA in birds, the parasite was only detected in black kite (33.3 %; 1/3); Eurasian jay (21.7 %; 5/23); black-billed magpie (15.2 %; 5/33), and griffon vulture (1 %; 1/105). It has also been detected in rats (16.7 %; 1/6) from Barcelona province, raccoons (3.6 %; 7/194) in Madrid province and American mink (9.2 %; 11/120) in north-eastern Spain.

3.6. Incidence of clinical toxoplasmosis in humans

Human incidence data expressed as hospitalisation rate (cases per 100,000 inhabitants) were presented in Table S3. The highest incidence rates were found in provinces within BR2 such as Burgos (1.99) and Lérida (1.29), followed by Soria (1.38) within BR4 and Valencia (1.20) within BR5.

Table 2
Pooled seroprevalence of *Toxoplasma gondii* exposition in wildlife from Spain.

Bioregion	Seroprevalence % (95 % CI)	Heterogeneity		
		I^2	τ^2	P value
BR1	36 (33–39)	93 %	2.53	<0.01
BR2	35 (33–37)	98 %	1.54	<0.01
BR3	19 (18–20)	97 %	1.41	<0.01
BR4	16 (13–20)	84 %	0.56	<0.01
BR5	29 (27–30)	95 %	2.65	<0.01

Taxonomic group	Seroprevalence % (95 % CI)	Heterogeneity		
		I^2	τ^2	P value
Ungulates	21 (20–22)	94 %	0.8459	<0.01
Carnivores	74 (71–76)	80 %	0.57	<0.01
Lagomorphs	14 (11–16)	28 %	0	0.25
Birds	41 (35–47)	99 %	3.47	<0.01

Global	Seroprevalence % (95 % CI)	I^2	τ^2	P value
Common effect model	24 (23–25)	97 %	1.88	<0.01

Table 3
Final generalized linear model (GzLM) explaining anti-*Toxoplasma gondii* seroprevalence in wild animals in Spain. Bolded values represent statistically significant associations.

Variables	Estimate ± standard error	z-Value, P
Intercept	−9.58 ± 1.57	−6.11[3]
Wetland	0.58 ± 0.05	**11.53**[3]
Unvegetated	0.42 ± 0.04	**10.38**[3]
Shrub	−0.14 ± 0.01	−14.66[3]
Permanent crops	−0.04 ± 0.01	−8.62[3]
Mixed forest	−0.27 ± 0.04	−6.94[3]
Irrigated arable	−0.30 ± 0.01	−21.07[3]
Heterogeneous agricultural areas	−0.16 ± 0.01	−16.23[3]
Grassland pastures	−0.16 ± 0.01	−13.00[3]
Broad-leaved forest	−0.03 ± 0.01	−4.29[3]
Artificial areas	−0.26 ± 0.03	−10.15[3]
Agroforestry areas	−0.18 ± 0.01	−17.94[3]
Bio 3	0.36 ± 0.03	**11.09**[3]
Bio 8	0.05 ± 0.02	**2.06**[1]
N ° intermediate hosts	0.14 ± 0.05	**2.78**[2]

[1] <0.05.
[2] <0.01.
[3] <0.001.

3.7. Environmental factors associated with the frequency of exposition to *Toxoplasma gondii* in animals and record of human toxoplasmosis requiring hospitalisation

Table 3 shows the abiotic variables which were retained in the best animal toxoplasmosis seroprevalence model. The wetland, unvegetated, Bio 3, Bio 8 and number of intermediate host species were all positively associated with increased exposure to *T. gondii*. Variables including shrub, permanent crops, mixed forest, irrigated arable, heterogeneous agricultural areas, grassland pastures, broad-leaved forest, artificial and agroforestry areas had no effect on the presence of the parasite.

Regarding hospitalisation due to toxoplasmosis in humans, only the abiotic variables permanent crops and Bio 8 showed a positive association (Table 4). Both the seroprevalence in wildlife and the population density did not show any effect on the hospitalisation rates by toxoplasmosis in humans.

Table 4
Final generalized linear model (GzLM) explaining hospitalisation rates in humans due to toxoplasmosis. Bolded values represent statistically significant associations.

Variables	Estimate ± standard error	z-Value
Intercept	−12.15 ± 0.21	−58.06[2]
Permanent crops	−0.01 ± 0.01	**11.53**[1]
Bio 8	0.45 ± 0.02	**2.34**[1]

[1] <0.05.
[2] <0.001.

4. Discussion

Spain, as a major part of the Iberian Peninsula in southern Europe, features a high spatial heterogeneity with high climatic diversity. Its geographical locations, between the Afrotropical and Palaearctic realms, make the Iberian Peninsula a privileged territory for wildlife and a European biodiversity hotspot (Rosso et al., 2018). The great diversity of the Iberian fauna and its role in the ecology/epidemiology of pathogens of public health relevance is well known (González-Barrio et al., 2022; Barasona et al., 2019).

The information gathered from this systematic review indicates that *T. gondii* is widespread in wildlife throughout Spain. Although numerous studies have investigated the degree of exposure to *T. gondii* of Spanish wildlife in the last two decades, no attempts have been carried out to jointly analyse the generated data in an integrative manner. This task would be essential to better understand the epidemiology of the parasite in its potential sylvatic reservoirs and to identify infection hotspots that might represent a public health threat.

Disease diagnosis in wildlife is difficult when compared to domestic animals. Detecting infected wildlife is extremely difficult and, if targeted passive surveillance is applied, the detection of infected wildlife requires a great deal of financial and human effort (Barroso et al., 2024). An important limitation for the diagnosis of infection in free-living animals is the lack of specific validated diagnostic tests (*e.g.*, serological techniques) which have specific validation requirements. Therefore, the use of non-validated serological techniques makes the interpretation of results difficult and could lead to misdiagnosis (López-Ureña et al., 2023a, 2023b). Information generated in the present review was mainly based on serological data generated from assays (DAT, ELISA, IFAT and MAT) that in most instances were not validated for all the wild species studied; consequently, the data obtained might not be showing the correct picture.

4.1. Geographical-environmental variables and human activity implication in the exposure to Toxoplasma gondii

This review highlights a widespread exposure to *T. gondii* in Spanish wildlife. However, the spatial distribution of this parasite was not homogenous, with significantly higher rates of anti-*T. gondii* antibodies in northern Spain (BR1 and BR2; 34–36 %) compared with those reported in the peninsular hinterland (BR3 and BR4; 17–18 %) (Fig. 1). This finding is in accordance with the geographical differences observed in previous studies of wild ruminants in different regions of Spain (Gauss et al., 2006; Gamarra et al., 2008; Castro-Scholten et al., 2021). Additionally, a north-south gradient of *T. gondii* seropositivity has also been reported in Scandinavian countries such as Finland (Jokelainen et al., 2010) and in Sweden (Ljungström et al., 1995).

The geographical differences in seroprevalence observed in the present review could be related to the type of habitat, presence/abundance of domestic/feral or wild felids and other intermediate hosts and environmental factors that might influence the persistence of viable *T. gondii* oocysts in each BR (Yan et al., 2016; Afonso et al., 2007) (Table 3). In the other hand, detailed and harmonised data collection results are of great interest for enabling direct comparison of results among different areas. Thus, the lack of harmonization procedures for data collection from different animal species, populations under study, sample sizes, and diagnostic method used may hinder the comparison of results among studies. Despite the remarkable degree of heterogeneity observed, the frequency of exposure to *T. gondii* in wildlife was also high and widespread; therefore, unravelling the most common route of infection would be highly desirable to design appropriate intervention strategies. BR1 and BR2 are characterized by mild temperatures and high average annual rainfall, which provide high humidity conditions. In contrast, BR3 and BR4 are regions with more pronounced temperature changes, low altitude mountains and cold winters, hot and dry summers, and rainy autumns and springs. Climatic conditions of BR1 and BR2, and to a lesser extent BR5, seem to be more appropriate for the survival and sporulation of *T. gondii* oocysts in soil, vegetables, and water bodies. All these environmental matrices can be contaminated with domestic or wild feline faeces, representing potential sources of infection for animals and humans. The capability of *T. gondii* oocysts to persist for long period at cold temperature (−10 °C–4 °C) in the environment and to retain their infectivity is now well established (Dubey, 1998). This means that oocysts can remain viable in irrigation water or soil for many months (Frenkel et al., 1975; Dubey, 1998; Lindsay et al., 2002; Lindsay and Dubey, 2009; ANSES, 2021). In this study, environmental, geographical, and anthropic variables such as wetland, isothermality, and mean temperature during wettest quarter (Table 3) were positively associated with a higher likelihood of *T. gondii* exposure in wildlife. All these variables were related to the optimal temperature and humidity conditions for the survival of *Toxoplasma* oocysts (Yan et al., 2016). A recently published worldwide review about the prevalence of *T. gondii* in wildlife describes that factors such as human density and environmental conditions contribute to the prevalence of the parasite in wildlife, and also focuses on the distribution and dispersal of the domestic cat as primary source of environmental contamination with infective oocyst and increased exposure to the parasite (Wilson et al., 2021). Thus, these variables can be considered risk factors for *T. gondii* exposure. Similarly, the dynamics of oocysts in the environment are influenced by seasonal rainfall, with storm run-off contributing to the transport of oocysts from land to wetlands and water bodies intended for human consumption, potentially leading to waterborne toxoplasmosis (Karanis et al., 2013). For example, the emergence of *T. gondii* infections in river otters has been associated with the higher density of human populations and the increased presence of feral and domestic cats (Gaydos et al., 2007; Shapiro et al., 2019a, 2019b; Miller et al., 2023). Likewise, we found that aquatic mammals such as American mink had a higher seroprevalence of *T. gondii* compared to terrestrial species.

4.2. Impact of the presence of domestic, feral and wild felids

In addition to environmental conditions favourable to the maintenance of *T. gondii* viability/infectivity in the environment, some human-driven factors may contribute to the increased prevalence of the parasite in the affected regions. This is the case of the presence of domestic, feral and wild felids, which can also affect the epidemiology of *T. gondii* (Gauss et al., 2003; Miró et al., 2004). In previous studies, an increase in feral cat populations, together with a human-driven decline of the Tasmanian devil, led to increased risk of exposure to *T. gondii* in naïve, native marsupials in Tasmania, Australia (Hollings et al., 2013), also, the prevalence in wild boar is influenced by the presence of cats in France (Beral et al., 2012). In addition, agent-based models analysing the spatial distribution of landscape structures in rural settings have suggested that humans and animals might have a higher risk of exposure to *T. gondii* oocyst due to the high density of cats in farm buildings (Gotteland et al., 2014a, 2014b). In Spain, human settlements and villages are spread out in forest areas throughout the northern region (Atlas Nacional de España, 2019). This dispersion of human settlements could also be accompanied by the spreading of domestic cats, which are often abundant and present as pets or stray cats (Zhu et al., 2023). According to previous studies, higher *T. gondii* seroprevalence rates have been reported in free-roaming cats than in household cats in northeast and north-central Spain (Gauss et al., 2003; Miró et al., 2004; Villanueva-Saz et al., 2022; Candela et al., 2022). Hence, contact of wildlife with felids in the surroundings farms and villages of those regions is more probable than in central areas of Spain, where stray cats are scarcer (Ferreras et al., 2021).

Other wild felids, like wildcats and lynxes, could be also involved in the cross-species transmission of *T. gondii* in wildlife (Sobrino et al., 2007; Candela et al., 2019; García-Bocanegra et al., 2010; Matas Méndez et al., 2023). The distribution of the wildcat in Spain is poorly understood, limiting our knowledge on the current status of *T. gondii* infection in this host (Sobrino et al., 2007; Candela et al., 2019; Millán et al., 2009). Seroprevalence rates over 50.0 % have been documented, suggesting that wildcats are also relevant hosts in the epidemiology of *T. gondii*. A recent study conducted in the centre of the Iberian Peninsula (BR3) has revealed low densities of wildcat populations (Ferreras et al., 2021), which is in agreement with the lower *T. gondii* seroprevalence rates (17–18 %) in wildlife in central Spain (BR3 and BR4) observed in this review. On the other hand, wildcats seem to be more frequent in the Spanish Mediterranean coast (Soto and Palomares, 2014; Gil-Sánchez et al., 2020), which is also in accordance with the moderated *T. gondii* exposure rates (28.9 %) observed in wildlife in southern and eastern Spain (BR5). Moreover, it is estimated that wildcat densities are higher in northern Spain (Lozano et al., 2007; Vázquez García et al., 2017), coinciding also with the higher seroprevalence rates (34–36 %) in wildlife in this area (BR1-BR2). Another felid species that could be involved in the epidemiology of *T. gondii* is the endangered Iberian lynx (García-Bocanegra et al., 2010; Matas Méndez et al., 2023). Studies conducted in the last 15 years have shown that *T. gondii* seroprevalence is high in Iberian lynxes, with rates ranging from 45.0 % to 80.8 % (Roelke et al., 2008; Sobrino et al., 2007; Millán et al., 2009; García-Bocanegra et al., 2010; Matas Méndez et al., 2023). Despite the increase in their numbers in recent years (Delibes-Mateos et al., 2022), it does not seem likely that the lynx has a significant effect on oocyst dispersal and contamination in the environment.

4.3. Public health risk

The main risk factors for *T. gondii* infection may be summarised as consumption of raw or undercooked meat, consumption of inadequately sanitized raw or fresh vegetables, blood transfusion, organ transplantation where these may contain viable tissue cysts, as well as handling cat faeces (Attias et al., 2020). A recent study has described the evolution of the most-probable main sources of infection in outbreaks of toxoplasmosis in humans in recent decades (Pinto-Ferreira et al., 2019). The authors reported on the apparent temporal progression of likely sources of infection and routes of transmission of global outbreaks of human toxoplasmosis described in published articles, and suggested a possible change in the epidemiology of reported toxoplasmosis outbreaks, with the primary source of infection changing from cysts in raw or undercooked meat to oocysts in fruits and vegetables. Because of this change, the authors suggested that more attention should be paid to the sanitation of vegetables, as well as to the quality of water used for drinking and irrigation purposes. Notwithstanding this change, clinical outbreaks of toxoplasmosis are continuously reported after consumption of raw or undercooked venison (Gaulin et al., 2020; Schumacher et al., 2021; Dubey et al., 2021; Conrady et al., 2022). Spain is one of the countries in Europe with the largest number of wild species hunted with near 20 million total captures per year (Sevillano Morales et al., 2018; MAPA, 2021). In terms of game meat consumption, a survey-based study showed that one out of three Spaniards had consumed game meat in the past year, assuming only 2 % of meat consumption, being much higher in rural areas than in urban areas (Lizana et al., 2022; ASICCAZA, 2023). In this review, we found elevated seroprevalence rates in wild ungulates, with at least one out of every four animals tested seropositive. This fact, together with the observation that the isolation rate of viable parasites is positively associated with MAT titres in ruminants (Dubey et al., 2020; Dubey et al., 2021) indicate that wild ungulates may be considered an excellent indicator of environmental contamination with *T. gondii* oocysts by definitive host species and a source of infection with public health significance.

On the other hand, the genetic types detected in wildlife (Type I, II and III) have been described also in humans in Europe. However, molecular data available are outdated and there is a remarkable lack of consensus and harmonization of *T. gondii* genotyping methods (Fernández-Escobar et al., 2022).

Prevention of human toxoplasmosis would only be effective if considered under an integrated and coordinated “One Health” approach. More efforts should be carried out to develop and enhance collaborations among parasitologists, physicians, veterinarians, ecologists, epidemiologists, and statisticians. Thus, it is necessary to establish monitoring and surveillance programmes for wildlife populations, especially those whose meat is consumed and have public health relevance. On the other hand, it would be also necessary to control feral cat populations, which represent an important risk factor for the transmission of the parasite. The management of wildlife-human contact in areas where populations overlap requires efforts to prevent the transmission of zoonotic pathogens including *T. gondii* and, finally, to raise public awareness of zoonotic diseases transmitted by wildlife.

Our study had some limitations. One of them is that the temporal distribution of the sampling, from 1989 and 2021, was not homogenous. Another limitation was that it was not possible to reach enough sample size for certain species within each bioregion considered, leading to imprecise estimates. Therefore, the seropositivity obtained at species level at each BR should be interpreted with caution. Serological techniques need prior validation for use in wildlife, as most diagnostic tests are primarily intended for domestic animals and humans (González-Barrio and Ruiz-Fons, 2019). Most of the data collected in this systematic review result from studies where small convenience sampling was applied. Therefore, the reported prevalence estimates may not be representative of the entire population of each of the wildlife species investigated due to selection bias. For example, in domestic animals (and humans), seroprevalence is known to increase with age as a result of longer exposure (Boughattas et al., 2016). Another limitation would be the distribution by bioregions and not by grids (more precise) of cases in wildlife and humans and, geographic data needs to be accurate in both animals and humans to obtain more robust results, grouping species by taxonomic groups may lead us to overlook the different diets of each species within the taxonomic group, such as scavenging birds or omnivorous ungulates, and finally, and most importantly, the absence of distribution/census data for domestic cats, cat colonies and feral cats, which are basically the main responsible for the excretion of *T. gondii* oocysts.

5. Conclusions

The information presented in this systematic review indicates that *T. gondii* is widespread in wildlife throughout Spain, showing a significantly higher seroprevalence in northern of the country compared to that reported in the hinterland, being more prevalent in carnivores than herbivores. It also shows the impact and presence of the parasite in domestic, feral, and wild felids and the relationship to favourable environmental conditions to maintain the viability of *T. gondii* oocysts. However, there is a substantial gap in research and insufficient knowledge, especially in areas where the human-livestock-wildlife interface overlaps, which prevents determining its impact and distribution in different habitats. This insufficient knowledge also hinders our understanding of the specific role that wildlife reservoirs play in the epidemiology of *T. gondii*, as well as the direct or indirect implications for public health. In addition, the gap in environmental contamination studies is even more critical and timely. This knowledge would certainly contribute to improve surveillance, prevention and control strategies against animal and human toxoplasmosis.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data are included in the supplementary material

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