A systematic review, meta-analysis and meta-regression of the global prevalence of *Toxoplasma gondii* infection in wild marine mammals and associations with epidemiological variables

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Abstract

Toxoplasma gondii infection in wild marine mammals is a growing problem and is associated with adverse impacts on marine animal health and public health. This systematic review, meta-analysis and meta-regression estimates the global prevalence of *T. gondii* infection in wild marine mammals and analyzes the association between *T. gondii* infection and epidemiological variables. PubMed, Web of Science, Science Direct, China National Knowledge Infrastructure, and Wanfang Data databases were searched until 30 May 2021. Eighty-four studies (n = 14,931 wild marine mammals from 15 families) were identified from literature. The overall pooled prevalence of *T. gondii* infection was 22.44% (3,848/14,931; 95% confidence interval (CI): 17.29% – 28.04%). The prevalence in adult animals 21.88% (798/3119; 95% CI: 13.40 –31.59) was higher than in the younger age groups. North America had a higher prevalence 29.92% (2756/9243; 95% CI: 21.77 – 38.77) compared with other continents. At the country level, the highest prevalence was found in Spain 44.26% (19/88; 95%CI: 5.21 – 88.54). Regarding climatic variables, the highest prevalence was found in areas with a mean annual temperature >20℃ 36.28% (171/562; 95% CI: 6.36 – 73.61) and areas with an annual precipitation >800 mm 26.92% (1341/5042; 95% CI: 18.20 – 36.59). The subgroup and meta-regression analyses showed that study-level covariates, including age, country, continent, and mean temperature, partly explained the between-study heterogeneity. Further studies are needed to investigate the source of terrestrial to aquatic dissemination of *T. gondii* oocysts, the fate of this parasite in marine habitat and its effects on wild marine mammals.

Keywords: Marine mammal; meta-analysis; *Toxoplasma gondii*; Toxoplasmosis; Worldwide

1. Introduction

Toxoplasma gondii is a worldwide protozoan parasite, which is highly prevalent in human population. This water-borne and food-borne zoonotic parasite has a broad

intermediate host range and can infect many terrestrial and marine mammals (Dubey, 2010). Despite its complex life cycle, felines are the only definitive hosts of *T. gondii*. Infected cats excrete thousands of environmentally resistant oocysts with their feces, which contaminate the environment (Elsheikha et al., 2020; Frenkel et al., 1975; Yilmaz et al., 1972). Surface runoff can carry *T. gondii* oocysts from land to ocean (Poulle et al., 2021; Conrad et al., 2005; VanWormer et al., 2014; Shapiro et al., 2015). *T. gondii* oocysts is widely distributed in the marine environment due to its strong adaptability. These oocysts can survive for up to 24 months in seawater under laboratory conditions and can sporulate in seawater and remain infectious to mice for months (Lindsay and Dubey, 2009).

The influx of *T. gondii* oocysts from land to sea can have a negative impact on marine mammals (Fayer et al., 2004; Poulle et al., 2021). Marine invertebrates can trap and retain *T. gondii* oocysts via filtering seawater or ingesting seaweed (Mazzillo et al., 2013) and migratory filter-feeding fish can retain infective oocysts in their digestive tract (Massie et al., 2010). These filter feeders are common food sources of marine mammals (Cabezón et al., 2011; Obusan et al., 2015) and thus represent an important source of infection by *T. gondii* oocysts (Mazzillo et al., 2013; Poulle et al., 2021). *T. gondii*-infected marine mammals manifest as meningoencephalitis, multiple organ necrosis, and hemorrhage (Lapointe et al., 1998; Roe et al., 2013). Abortion, attributed to toxoplasmosis, has been reported in a sea otter from California (Shapiro et al., 2016). Toxoplasmosis is also a common cause of mortality of sea otters and Hawaiian monk seals (Dubey, 2010; Dubey et al., 2020; Miller et al., 2020).

T. gondii is one of the most common food-borne pathogens (Wei et al., 2021). In countries such as Japan and Faroe Islands, wild marine mammals, such as small cetaceans

(e.g., whales and dolphins) are used as food sources (Vail et al., 2020) and local communities are at risk of acquiring infection via ingestion of uncooked meat of marine mammals harboring *T. gondii*. Serological evidence of *T. gondii* infection in wild marine mammals has been reported in the Pacific Rim (Burgess et al., 2018), Southern Ocean (Núñez-Egido et al., 2020), Philippine Islands (Obusan et al., 2019) and Sub-Antarctic area (Michael et al., 2016), which raises concerns of the adverse impact of *T. gondii* infection on the health of marine mammals and humans. Previous studies have brought new insights to *T. gondii* epidemiology and its negative impact on wild marine mammals and public health, highlighting the need to generate more data and tools to understand the extent of and variables associated with *T. gondii* infection in wild marine animals. This knowledge is pivotal for understanding of the transmission dynamics and monitoring of *T. gondii* infection within and among wild marine mammals.

In the present systematic review and meta-analysis, we identified the global pooled prevalence of *T. gondii* infection among wild marine mammals. In addition, we investigated the correlation between *T. gondii* infection and epidemiological variables, including year of publication, detection method, age, gender, habitat, level of economic status, geographical information (continent and country), and climate factors (temperature and precipitation).

2. Materials and methods

This study was conducted in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) reporting guideline (Moher et al., 2009; **Table S1**).

2.1. Literature search strategy

We searched five electronic databases, including the Web of Science, Medline, Science Direct, China National Knowledge Infrastructure (CNKI), and Wanfang Data for articles published until 30 May 2021. The search was performed using a combination of Medical Subject Heading (MeSH) terms (e.g., “Dolphin”, “Trichechus”, “Otters”, “Porpoises”, “Sea Lions”, “Seals, Earless”, “Whales”, “Dugong”, “*Toxoplasma*”) and text (e.g., “Marine mammal” and “Polar bear”). The retrieval formulas are shown in **Table S2**. Additionally, we searched reference lists in the identified studies for additional relevant studies. We did not apply any restrictions regarding the date of publication or geographical location. However, search was focused only on English language articles.

2.2. Article selection criteria

Studies were eligible if they are related to *T. gondii* infection in wild marine mammals, reported the prevalence of *T. gondii* infection, results are based on individual rather than pooled samples, and cross-sectional studies published up to 30 May 2021. Studies were excluded if they did not provide enough or original information, case reports, review papers, conference abstracts, other non–full-length articles, conducted on captive animals, and non–English language publications. The identified articles were managed using EndNote (Version: X9 Clarivate Analytics, USA).

2.3. Study selection and data extraction

After two authors (M-Y. L., X-N. G.) screened titles and abstracts for initial eligibility, J-Y. M. and W.C. independently reviewed the full texts of potentially eligible articles for inclusion and exclusion criteria. Any disagreements were resolved by consulting H.E. The selected studies were reviewed for type of study, publication year, detection method, marine mammal species, habitat, gender, age, economic development level, and geographic information (continent, and country). In addition, information on mean annual temperature and annual precipitation was obtained from the National Centers for Environmental Information (https://gis.ncdc.noaa.gov/maps/ncei/cdo/monthly/). All extracted information was recorded in Microsoft Excel 2019 (Microsoft, Redmond, WA, USA).

2.4. Study quality assessment

The quality of the included studies was assessed independently by two authors (M-Y L, X-N G) using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) method (Guyatt et al., 2008; Wang et al., 2021). The quality assessment included five criteria: clarity of the study aim, study contains information on the gender or age, detection method is clearly described, a sample size > 100, and known sampling time. Each criterion was represented by 1 point. The study that met the five criteria were given a score of 5 points. Based on this analysis, studies were rated as having a low quality (0–1 point), moderate quality (2–3 points) and those with 4–5 points were considered high quality (Gong et al., 2020a).

2.5. Statistical analysis

All data analyses were performed using R-based software v3.6.3 ("R core team, R: A language and environment for statistical computing", R core team 2018). The R codes used for data analysis are shown in **Table S3**. To fit the data to Gaussian distribution before meta-analysis, four conversion methods were used, including logarithmic conversion (PLN), logit transformation (PLOGIT), arcsine transformation (PAS), and double-arcsine transformation (PFT). Data normality was checked by Shapiro-Wilk test. The closer the 'W' is to the value 1 (maximum value of this statistic) and p values $\geqslant$ 0.05, the closer the fit to Gaussian normal distribution.

Due to the anticipated heterogeneity between and within studies, we used a random effects model which assumes that the true effect is not the same across studies. We calculated the Cochran Q value and the inconsistency I^2 statistic. The between-study heterogeneity was deemed significant if the p value of the Q test was < 0.10, or if the I^2 statistic was > 50%. The result of meta-analysis was visualized using a forest plot. The Egger's bias test at $p < .05$ and the funnel plot asymmetry were used to detect any publication bias. The influence of each included study on the meta-analysis results was examined using the leave-out-one approach, where one study was deleted at a time from the meta-analysis and other studies were analyzed (Gong et al., 2020b; Wang et al., 2021; Wei et al., 2021).

To investigate the source of heterogeneity, we performed meta-regression and subgroup meta-analyses, based on study-level characteristics as potential explanatory sources, including year of publication (1997-2010 versus after 2010), detection method (immunohistochemical versus serological, and nucleic-acid based), age (pups versus juveniles and adults), gender (female versus male), habitat (aquatic versus subaquatic and terrestrial habitats), and level of

159 economic development (developed versus developing countries). In addition, we used the
160 publication year as a covariate and related variables for joint analysis to explain some of the
161 heterogeneity caused by publication year, and R^2 represented heterogeneity explained by the
162 covariate (Wang et al., 2021; Wei et al., 2021).

163 Given the global scale of the meat-analysis and the anticipated variations between
164 geographical regions and climatic conditions, we extended the subgroup and meta-regression
165 analyses to geographical region and climatic factors. This included country (Canada versus 7
166 other countries), continent (Antarctica versus other continents), mean annual temperature
167 (<0°C versus higher temperatures), and annual precipitation (< 200mm versus higher
168 precipitation levels). Reliable estimation of the between-study heterogeneity using the
169 random-effects model requires many studies (Guolo, 2012). Therefore, meta-regression
170 analysis of the epidemiological variables was only conducted when 3 or more studies were
171 available.

172

173 **3. Results**

174 **3.1. Characteristics of the included studies**

175 Searching five electronic databases and identification of the eligible studies were
176 performed using the selection strategy outlined in Figure 1. We identified 2,680 reports, of
177 which 84 studies met eligibility criteria and were included in the analyses. Summary
178 characteristics of the included studies are shown in **Table 1**. One of the eligible studies was
179 obtained from the reference list of White et al. (2013). According to the quality criteria, 59
180 studies were rated as having high quality and 25 were of moderate quality.

3.2. Risk of publication bias and sensitivity analysis

The conversion result of PAS was closer to normal distribution (**Table S4**), and therefore we chose the combination result of PAS conversion for meta-analysis. The use of the random-effects model was appropriate for the analysis because between-study heterogeneity was significant (I^2 = 98%) (**Figure 2**). The funnel plot showed that all studies are distributed symmetrically, indicating no evidence of publication bias (**Figure 3**). The results of Egger's test further confirmed the lack of evidence of publication bias among the included studies (p = 0.9134, **Figure 4, Table 2**). The sensitivity analysis indicated that there was no significant effect on the meta-analysis findings after omitting one study at a time, indicating that the results are reliable (**Figure 5**).

3.3. Primary outcome

The primary outcome of this systematic review and meta-analysis was to assess the prevalence of *T. gondii* infection in wild marine mammals from a global perspective. The study included 14,931 wild animals from 15 families. The overall pooled prevalence of *T. gondii* infection in the wild marine mammals was 22.44% (3848/14931, 95% confidence interval (CI): 17.29%-28.04%; **Figure 2**). We compared the prevalence of *T. gondii* infection among the 15 families of marine animals studied. Roughly equal pooled prevalence was detected in Balaenopteridae 13.9% (4/215; 95% CI: 0.00-67.83), Odobenidae 14.37% (18/126; 95% CI: 4.30-28.46), Otariidae 13.99% (591/2185; 95% CI: 2.46-31.90), Phocidae 15.72% (595/4097; 95% CI: 9.31-23.34), and *Phocoenidae* 15.30% (65/262; 95% CI: 0.00-

47.99). The prevalence of *T. gondii* infection in other animal families is shown in **Table 3**.

3.4. Secondary outcome

The subgroup and meta-regression analyses revealed that four covariates, including age (**Table 4**), country, continent and mean temperature (**Table 5**), can partly explain between-study heterogeneity ($p < 0.05$). In the age subgroup, the prevalence of *T. gondii* infection in adult animals 21.88% (798/3119; 95% CI: 13.40-31.59) was higher compared with the prevalence of the two younger age groups (**Table 4**). Of all continents, North America had the highest prevalence with 29.92% (2756/9243; 95% CI: 21.77-38.77). Of all countries, the highest prevalence was found in Spain 44.26% (19/88; 95% CI: 5.21-88.54), followed by USA 30.93% (2459/7720; 95% CI: 21.54-41.19), and United Kingdom 25.28 % (415/1390; 95% CI: 14.41-38.02). Regarding the climatic factors, the prevalence in areas with annual mean temperature >20°C (36.28%; 95% CI: 6.36-73.61; 171/562) was significantly higher compared with other areas with lower temperatures (**Table 5**).

The prevalence in areas with annual precipitation >800mm (26.92%; 95% CI: 18.20-36.59; 1341/5042) was higher than other areas with a lower precipitation (**Table 5**). However, the differences were not statistically significant ($p = 0.085$). No significant difference ($p = 0.848$; **Table 4**) was detected in the prevalence between developed countries 21.98% (3392/12793; 95% CI: 16.53-27.97) and developing countries 20.63% (256/1140; 95% CI: 8.00-37.23). Likewise, publication year, detection method, gender, and habitat did not have any significant influence on the prevalence of *T. gondii* infection in wild marine mammals.

Four types of methods used for detecting *T. gondii* infection in wild marine mammals

were reported in the 84 analyzed studies, including bioassay, immunohistochemical, serological and nucleic acid-based. As shown in **Table 1**, the primary testing method was serological, followed by nucleic acid-based amplification, immunohistochemical method, and bioassay. Serological methods, included latex agglutination test (LAT), modified agglutination test (MAT), direct agglutination test (DAT), indirect fluorescence antibody test (IFAT), dye test (DT), enzyme linked immunosorbent assay (ELISA), indirect hemagglutination assay (IHA), and immunoblotting assay. Nucleic acid-based amplification methods included nested Polymerase Chain Reaction (nested PCR), Reverse Transcription-polymerase Chain Reaction (RT-PCR), and quantitative real-time Polymerase Chain Reaction (qPCR).

4. Discussion

The global pooled prevalence of *T. gondii* infection in wild marine mammals was 22.44%, supporting growing evidence that *T. gondii* infection is widespread in wild marine mammals. *T. gondii* prevalence varied considerably, from 3.24% to 65.08%, between wild marine mammal families. The factors responsible for this large variation in the reported *T. gondii* prevalence is not known but is likely to be related to different detection methods and different feeding habits of marine mammals. Our analysis included 15 families of marine mammals with different prey choices, which can influence the risk of exposure to *T. gondii* infection (Johnson et al., 2009).

Marine mammals with high *T. gondii* prevalence rates, such as Odobenidae and Ursidae (polar bears) prey on seals and whales (Gjertz & Øystein, 1992; Thiemann et al., 2008;

247 Mckinney et al., 2017), and are more likely to be infected via consumption of prey tissue
248 containing *T. gondii*. The low prevalence rates 4.39% and 6.23% detected in herbivore marine
249 mammals Dugongidae and Trichechidae, respectively, may be attributed to limited exposure
250 to infection via ingestion of oocysts from contaminated seawater or seagrass (Wong et al.,
251 2020; Wyrosdick et al., 2017). The combined prevalence in Mustelidae (mainly river otters
252 and sea otters) was 40.59%. Marine invertebrates particularly mollusks are a common food
253 source for these animals (Miller et al., 2002a; Miller et al., 2020). Since marine bivalve
254 shellfish can filter and retain *T. gondii* oocysts from seawater (Coupe et al., 2018; Cong et al.,
255 2019; Cong et al., 2021), and they provide more opportunities for exposure of Mustelidae to
256 *T. gondii* (Krusor et al., 2015).

257 The particularly high prevalence detected in Iniidae (65.08%) may be related to the type
258 of aquatic environment. The Iniidae (Amazon River dolphin) is the only freshwater
259 mammalian group identified in our analysis. Surface runoff contaminated with cat feces
260 facilitates the spread of *T. gondii* oocysts from land to the aquatic habitat of the Amazon River
261 dolphin, increasing the risk of infection. The lowest infection rate (3.24%) detected in
262 Physeteridae (sperm whales) is likely because whales inhabit the open ocean (Rendell &
263 Frantzis, 2016), which is less affected by surface runoff pollution. This observation is
264 consistent with a previous study (Hermosilla et al., 2016).

265 One of the limitations of meta-analysis of epidemiological studies is data heterogeneity,
266 which can be related to sampling frame (Ni et al., 2020; Wang et al., 2021) or different
267 detection methods (Gong et al., 2021). Subgroup analysis revealed no significant differences
268 between publication years or detection methods ($p > 0.05$, **Table 4**). Detection of *T. gondii*

infection has historically been achieved using a number of methods. Serological and immunohistochemical methods are useful, however cross-reactivity may lead to unreliable results (Miller et al., 2002b; Gong et al., 2021). Nucleic acid-based amplification methods can provide high detection rates and specific identification of *T. gondii* compared with other methods (Su et al., 2010). However, they are expensive, require specialized instruments, and do not distinguish between viable and non-viable oocysts (Dubey et al., 2020).

Although gender has been considered a potential risk factor in previous meta-analysis of *T. gondii* infection in cattle (Gong et al., 2020a) and goats (Wei et al., 2021), there was no significant difference in the prevalence between male and female wild marine mammals in our study. Regarding the habitat subgroup, the prevalence in subaquatic animals (23.51%) was nearly similar to that in aquatic animals (22.32%), whereas terrestrial animals had a lower prevalence (17.97%). In our study, the subgroup of terrestrial animals included polar bear, which is the largest terrestrial predator in the circumpolar region (Naidenko et al., 2013). It is unlikely for bears to become infected via surface runoff because of the limited presence of cats in the arctic. Also, the arctic regions have water masses that are relatively isolated from global ocean circulation, which further reduces the risk of water-borne infection by *T. gondii* oocysts. However, accumulation of *T. gondii* oocysts in estuarine environment during Spring may lead to pollution of the arctic coast, and thus increasing exposure to infection (Simon et al., 2013). Additionally, transmission of *T. gondii* between polar bears and other animal species can occur even in the absence of cats (Prestrud et al., 2010).

The economic development level had no significant effect on *T. gondii* prevalence. While this may initially suggest a lack of influence of the economic disadvantage in developing

291 countries on the prevalence of *T. gondii* infection in wild marine mammals, this trend was not
292 consistent. For example, Iran had a very high prevalence (83.33%) of *T. gondii* in wild marine
293 mammals compared with that reported in developed countries (Table 5). That said, results
294 based on one study in Iran can not represent the situation in developing countries.

295 Interestingly, meta-regression analysis identified age, country, continent, and annual
296 mean temperature as variables significantly associated with *T. gondii* infection in wild marine
297 mammals. The prevalence was significantly higher in adult marine mammals compared with
298 that of the juvenile and pup groups, suggesting age-dependent increase of *T. gondii* infection.
299 It is reasonable to assume that the longer an animal remains alive, the greater the risk of being
300 exposed to infection. A similar finding was previously reported in terrestrial mammals
301 (horses: Li et al., 2020; cattle: Gong et al., 2020a; goats: Wei et al., 2021; pigs: Foroutan et al.,
302 2019; wild boars: Rostami et al., 2017).

303 We noted differences in the biogeographic patterns of *T. gondii* prevalence. The highest
304 infection rate in wild marine mammals was detected in Spain (44.26%), followed by USA
305 (30.93%) and United Kingdom (25.28 %). At the continent level, the highest prevalence was
306 found in North America. Because felines are the only definitive host of *T. gondii* (Elmore et
307 al., 2010), differences in the prevalence between geographical regions may be related to
308 differences in the density of cats between various regions. A previous meta-analysis of *T.*
309 *gondii* infection in felids (Montazeri et al. 2020) showed that the highest infection rate of
310 domestic cats was found in Australia (52%), while the highest infection rate of wild felids was
311 detected in Africa (74%). Regional differences in prevalence may also be related to variations
312 in aquatic dispersion of *T. gondii* oocysts driven by ocean currents (**Figure 6**), which can

influence the distribution of *T. gondii* oocysts in seawater, resulting in different levels of *T. gondii* contamination in marine environment (Poulle et al., 2021). However, the effect of dispersion by ocean currents on the biogeography of *T. gondii* infection in marine environment remains to be elucidated.

We considered whether there is a relationship between *T. gondii* prevalence and climatic factors in each geographical region. The analysis revealed statistical differences between mean temperatures, with the highest (36.28%) and lowest (9.68%) prevalence associated with >20°C and <0°C, respectively. This result was consistent with a previous meta-analysis of chronic toxoplasmosis in pregnant women (Rostami et al., 2021). Temperature affects sporulation and infectivity of *T. gondii* oocysts (Shapiro et al., 2019).

The study used a robust methodology in accordance with the PRISMA statement to extensively search five electronic databases. However, the study has potential limitations. First, we did not search other bibliographic databases (e.g., Cochrane Library, Embase, Scopus) and, thus, our systematic review may not be representative of all relevant research in the field. Second, the relatively small number of the included studies and the high level of heterogeneity identified among studies should be considered when interpreting the results. Third, it remains to be confirmed whether the association between the identified variables and *T. gondii* infection in wild marine mammals is valid in populations not represented in our meta-analysis.

5. Conclusion

In this systematic review and meta-analysis, the worldwide pooled prevalence of *T.*

gondii infection in wild marine mammals was 22.44%. This result adds to the growing evidence base suggesting that *T. gondii* infection in wild marine mammals is not restricted to certain geographical regions, highlighting the adverse impact of *T. gondii* on marine ecosystem, marine animal health and public health. Further research is required to determine the prevalence of *T. gondii* infection in wild marine mammals in under-studied areas, particularly in Africa, Asia, Oceania, and South America. Future research is also needed to investigate the dissemination routes of *T. gondii* oocysts from land to sea and the effect of ocean currents on the fate of the parasite in marine environment. Our results consolidate current knowledge of *T. gondii* infection and associated epidemiologic characteristics in wild marine mammals. This knowledge is useful for predicting disease risk and for establishing measures to limit the impact of toxoplasmosis on wild marine mammals.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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Ethical approval

No ethical approval was required.

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Table 1. Summary of the main characteristics of the eligible studies on *Toxoplasma gondii* infection in wild marine mammals..

No.	Reference	No. positive/ Total examined	Marine mammals	Detection Method(s)	Study design	Quality score	Study quality[a]
	Africa						
1	Lane et al. (2014)	0/40	Delphinidae,	Immunohistochemical	Cross-sectional	4	High
2	Namroodi et al. (2018)	30/36	Phocidae	Serological	Cross-sectional	4	High
	Antarctica						
3	Jensen et al. (2012)	40/102	Otariidae Phocidae	Serological	Cross-sectional	4	High
4	Rengifo-Herrera et al. (2012)	28/211	Otariidae Phocidae	Serological	Cross-sectional	4	High
5	Tryland et al. (2012)	0/116	Otariidae Phocidae	Serological	Cross-sectional	5	High
	North America						

6	Tocidlowski et al. (1997)	46/103	Mustelidae	Serological	Cross-sectional	5	High
7	Cole et al. (2000)	15/67	Mustelidae	Bioassay	Cross-sectional	3	Middle
8	Mikaelian et al. (2000)	6/22	Monodontidae	Serological	Cross-sectional	3	Middle
9	Lambourn et al. (2001)	29/380	Phocidae	Serological	Cross-sectional	4	High
10	Miller et al. (2002a)	115/223	Mustelidae	Serological	Cross-sectional	4	High
11	Miller et al. (2002b)	65/243	Mustelidae	Serological	Cross-sectional	4	High
12	Dubey et al. (2003)	328/761	Mustelidae, Phocidae, Otariidae, Delphinidae, Odobenidae, Monodontidae	Serological	Cross-sectional	4	High
13	Hanni et al. (2003)	36/175	Mustelidae	Serological	Cross-sectional	4	High
14	Measures et al. (2004)	127/328	Phocidae	Serological	Cross-sectional	5	High
15	Miller et al. (2004)	35/219	Mustelidae	Serological	Cross-sectional	3	Middle
16	Dubey et al. (2005)	146/146	Delphinidae	Serological	Cross-sectional	5	High
17	Rah et al. (2005)	30/500	Ursidae	Serological	Cross-sectional	5	High
18	Littnan et al. (2006)	2/18	Phocidae	Serological	Cross-sectional	2	Middle
19	Aguirre et al. (2007)	2/67	Phocidae	Serological	Cross-sectional	3	Middle

20	Gaydos et al. (2007)	7/40	Mustelidae	Serological	Cross-sectional	3	Middle
21	Thomas et al. (2007)	17/344	Mustelidae	Serological	Cross-sectional	4	High
22	Dubey et al. (2008)	27/52	Delphinidae	Serological	Cross-sectional	3	Middle
23	Brancato et al. (2009)	18/30	Mustelidae	Serological	Cross-sectional	3	Middle
24	Johnson et al. (2009)	56/118	Mustelidae	Serological	Cross-sectional	4	High
25	Kirk et al. (2010)	18/136	Ursidae	Serological	Cross-sectional	5	High
26	Gibson et al. (2011)	85/161	Otariidae Phocidae Phocoenidae Delphinidae Physeteridae Mustelidae	Nucleic acid based	Cross-sectional	4	High
27	Goldstein et al. (2011)	6/163	Mustelidae	Serological	Cross-sectional	4	High
28	Hueffer et al. (2011)	0/116	Phocidae	Serological	Cross-sectional	4	High
29	Simon et al. (2011)	86/828	Phocidae	Nucleic acid based, Serological	Cross-sectional	5	High
30	Bossart et al. (2012)	1/28	Trichechidae	Serological	Cross-sectional	4	High
31	White et al. (2013)	29/30	Mustelidae	Serological	Cross-sectional	3	Middle
32	Greig et al. (2014)	14/283	Phocidae	Serological	Cross-sectional	4	High

33	Carlson-Bremer et al. (2015)	536/1630	Otariidae	Serological	Cross-sectional	4	High
34	Al-Adhami et al. (2016)	34/194	Phocidae, Odobenidae, Balaenidae, Monodontidae	Serological	Cross-sectional	4	High
35	Bauer et al. (2016)	0/34	Phocidae	Serological	Cross-sectional	4	High
36	Smith et al. (2016)	3/44	Trichechidae	Serological	Cross-sectional	3	Middle
37	Atwood et al. (2017)	33/138	Ursidae	Serological	Cross-sectional	4	High
38	Burgess et al. (2018)	184/710	Mustelidae	Serological	Cross-sectional	4	High
39	Verma et al. (2018)	65/70	Mustelidae	Serological	Cross-sectional	3	Middle
40	Bachand et al. (2019)	12/61	Phocidae	Serological	Cross-sectional	3	Middle
41	Reiling et al. (2019)	32/81	Phocidae	Nucleic acid based	Cross-sectional	3	Middle
42	Shapiro et al. (2019)	135/135	Mustelidae	Bioassay	Cross-sectional	4	High
43	Miller et al. (2020)	334/542	Mustelidae	Serological	Cross-sectional	4	High
44	Sanders II et al. (2020)	53/220	Mustelidae	Nucleic acid based	Cross-sectional	4	High
	South America						
45	Santos et al. (2011)	82/95	Iniidae	Serological	Cross-sectional	4	High
46	Sulzner et al. (2012)	8/112	Trichechidae	Serological	Cross-sectional	4	High

47	McAloose et al. (2016)	0/21	Balaenidae	Immunohistochemical	Cross-sectional	2	Middle
48	Costa-Silva et al. (2019)	3/185	Delphinidae	Immunohistochemical	Cross-sectional	5	High
49	Calvo-Mac et al. (2020)	1/19	Mustelidae	Serological	Cross-sectional	3	Middle
	Europe						
50	Oksanen et al. (1998)	0/645	Phocidae	Serological	Cross-sectional	4	High
51	Cabezón et al. (2004)	11/58	Phocoenidae Delphinidae	Serological	Cross-sectional	3	Middle
52	Sobrino et al. (2007)	6/6	Mustelidae	Serological	Cross-sectional	3	Middle
53	Alekseev et al. (2009)	46/221	Delphinidae Monodontidae	Serological	Cross-sectional	4	High
54	Forman et al. (2009)	8/101	Ziphiidae, Delphinidae, Phocoenidae, Balaenopteridae	Serological	Cross-sectional	4	High
55	Oksanen et al. (2009)	98/527	Ursidae	Serological	Cross-sectional	5	High
56	Guardo et al. (2010)	4/8	Delphinidae	Serological	Cross-sectional	4	High
57	Jensen et al. (2010)	163/704	Ursidae	Serological	Cross-sectional	5	High
58	Pretti et al. (2010)	3/4	Delphinidae	Nucleic acid based,	Cross-sectional	4	High

				serological			
59	Cabezón et al. (2011)	14/103	Phocidae	Serological	Cross-sectional	5	High
60	Chadwick et al. (2013)	108/271	Mustelidae	Serological	Cross-sectional	5	High
61	Naidenko et al. (2013)	2/26	Ursidae	Serological	Cross-sectional	4	High
62	Bernal-Guadarrama et al. (2014)	2/24	Delphinidae	Serological	Cross-sectional	3	Middle
63	Blanchet et al. (2014)	0/20	Phocoenidae	Nucleic acid based	Cross-sectional	4	High
64	Profeta et al. (2015)	10/70	Delphinidae, Balaenopteridae, Physeteridae, Ziphiidae	Serological	Cross-sectional	3	Middle
65	Hermosilla et al. (2016)	0/5	Physeteridae	Nucleic acid based	Cross-sectional	4	High
66	van de Velde. et al. (2016)	121/292	Mustelidae, Phocoenidae, Delphinidae, Balaenopteridae, Phocidae, Ziphiidae, Physeteridae,	Serological	Cross-sectional	4	High

67	Waap et al. (2016)	0/2	Mustelidae	Serological	Cross-sectional	3	Middle
68	Alekseev. et al. (2017)	11/78	Monodontidae	Serological	Cross-sectional	3	Middle
69	Santoro.et al. (2017)	0/6	Mustelidae	Nucleic acid based	Cross-sectional	4	High
70	Smallbone et al. (2017)	168/654	Mustelidae	Serological	Cross-sectional	5	High
71	Pintore et al. (2018)	7/45	Delphinidae, Balaenopteridae	Nucleic acid based	Cross-sectional	3	Middle
72	Scotter et al. (2019)	6/39	Odobenidae	Serological	Cross-sectional	4	High
73	Núñez-Egido et al. (2020)	0/70	Otariidae	Serological	Cross-sectional	4	High
74	Terracciano et al. (2020)	6/26	Delphinidae	Serological	Cross-sectional	3	Middle
	Asia						
75	Murata et al. (2004)	7/59	Delphinidae	Serological	Cross-sectional	3	Middle
76	Omata et al. (2006)	0/8	Delphinidae	Nucleic acid based	Cross-sectional	4	High
77	Fujii et al. (2007)	3/77	Phocidae	Serological	Cross-sectional	4	High
78	Obusan et al. (2015)	7/23	Delphinidae	Nucleic acid based	Cross-sectional	4	High
79	Obusan et al. (2019)	20/28	Delphinidae	Nucleic acid based	Cross-sectional	4	High
			Physeteridae, Balaenopteridae	Serological			

	Oceania						
80	Omata et al. (2005)	33/58	Delphinidae	Serological	Cross-sectional	3	Middle
81	Lynch et al. (2011)	0/104	Otariidae	Serological	Cross-sectional	5	High
82	Roe et al. (2013)	17/28	Delphinidae	Immunohistochemical	Cross-sectional	4	High
83	Michael et al. (2016)	3/50	Otariidae	Serological	Cross-sectional	4	High
84	Wong et al. (2020)	5/114	Dugongidae	Serological	Cross-sectional	5	High

a: The quality scores identified studies as high-quality (4-5 points), moderate-quality (2-3 points) and low-quality (0-1 point).

Table 2. Egger' s test result for publication bias.

slope	bias	se. bias	t	df	*p*-value
0.5032	-0.1685	1.5444	-0.11	82	0.9134

Table 3. Pooled prevalence and heterogeneity statistics of *T. gondii* infection in various families of wild marine mammals.

Family	No. of studies	No. examined	No. positive	% (95% CI)	Heterogeneity statistics		
					χ^2	*p*–value	I^2 (%)
Balaenidae	2	23	1	6.23 (0.00-79.38)	4.12	0.04	75.7
Balaenopteridae	6	215	4	13.90 (0.00-67.83)	24.97	<0.01	80.0
Delphinidae	21	1066	505	36.99 (15.63-61.06)	1090.72	<0.01	98.2
Dugongidae	1	114	5	4.39 (1.25-9.06)	-	-	-
Iniidae	2	96	82	65.08 (0.00-100.00)	3.72	0.05	73.1
Monodontidae	6	310	34	9.49 (2.15-19.91)	19.48	<0.01	74.3
Mustelidae	26	4574	1624	40.59 (28.90-52.80)	1443.56	<0.01	98.3
Odobenidae	3	126	18	14.37 (4.30-28.46)	7.32	0.03	72.7
Otariidae	9	2185	591	13.99 (2.46-31.90)	388.04	<0.01	97.9
Phocidae	22	4097	595	15.72 (9.31-23.34)	730.84	<0.01	97.1
Phocoenidae	5	262	65	15.30 (0.00-47.99)	67.54	<0.01	94.1
Physeteridae	7	32	3	3.24 (0.00-18.64)	6.86	0.33	12.5
Trichechidae	3	184	12	6.23 (2.94-10.45)	0.27	0.87	0.00
Ursidae	6	1555	285	18.05 (7.82-31.20)	161.50	<0.01	96.9
Ziphiidae	5	49	20	27.80 (2.43-61.73)	7.81	0.1	48.8

Abrreviations: CI = Confidence interval; x^2 = heterogeneity statistic. *p*-value < 0.05 is statistically significant; I^2 = heterogeneity index.

Table 4. Pooled prevalence, heterogeneity statistics, and meta-regression of epidemiological variables associated with *T. gondii* infection in wild marine mammals.

		No. ofstudies	No. examined	No. positive	% (95% CI)	Heterogeneity statistics			Univariate me	
						χ^2	*p*–value	I^2 (%)	*p*–value	Coefficient (
Publication year									0.211	0.085 (-0.048
	1997-2010	33	6448	1507	26.90 (18.23-36.56)	2123.97	0	98.5		
	After 2010	51	8483	2341	19.70 (13.47-26.78)	2798.30	0	98.2		
Detection method									0.111	-0.235 (-0.524
	Immunohistochemical	4	274	20	6.28 (0.00-31.49)	66.92	<0.01	95.5		
	Serological	72	13901	3485	22.75 (17.49-28.48)	4065.41	0	98.3		
	Neuclic acid-based	12	748	204	16.23 (4.31-33.78)	295.73	<0.01	96.3		
Age									0.043	-0.142 (-0.279
	Pup	23	764	96	2.94 (0.00-10.13)	144.59	<0.01	84.8		
	Juvenile	25	1235	222	12.39 (5.37-21.05)	209.66	<0.01	88.6		
	Adult	31	3119	798	21.88 (13.40-31.59)	919.22	<0.01	96.7		
Gender									0.935	0.007 (-0.154
	Female	28	2429	583	20.53 (11.56-31.02)	755.09	<0.01	96.4		
	Male	28	2506	620	19.10 (9.40-30.58)	741.02	<0.01	96.4		
Habitat									0.771	0.020 (-0.112
	Aquatic	34	2351	731	22.32 (10.66-36.78)	1959.44	0	98.3		
	Subaquatic	50	10982	2828	23.51 (17.17-30.51)	3216.31	0	98.5		

	Terrestrial	6	1555	285	17.97 (7.91-31.00)	162.99	<0.01	96.9		
Economic status									0.848	0.016 (-0.150
	Developed country	69	12793	3392	21.98 (16.53-27.97)	3934.03	0	98.3		
	Developing country	15	1140	256	20.63 (8.00-37.23)	533.20	<0.01	97.4		

Abrreviations: CI = Confidence interval; x^2 = heterogeneity statistic. p-value < 0.05 is statistically significant; I^2 = heterogeneity index; R^2: proportion of between-study variance explained.

Table 5. Pooled prevalence, heterogeneity statistics, and meta-regression of geographical and climatic factors associated with *T. gondii* infection in wild marine mammals.

		No. studies	No. examined	No. positive	% (95% CI)	Heterogeneity			Univariate meta-regression		
						χ^2	*p*–value	I^2 (%)	*p*–value	Coefficient (95% CI)	R^2 (%)
Country									0.0142	0.167 (0.033 to 0.300)	3.22
	Canada	7	1523	297	20.55 (10.09-33.56)	138.60	<0.01	95.7			
	Italy	6	159	30	21.07 (9.51-35.70)	15.71	<0.01	68.2			
	Japan	3	144	10	5.33 (0.65-14.12)	5.25	0.07	61.9			
	Norway	5	1596	255	7.40 (0.18-23.72)	302.22	<0.01	98.7			
	Russia	5	523	72	10.84 (5.10-18.39)	21.80	<0.01	81.7			
	Spain	3	88	19	44.26 (5.21-88.54)	32.11	<0.01	93.8			
	United Kingdom	5	1390	415	25.28 (14.41-38.02)	93.71	<0.01	95.7			
	USA	34	7720	2459	30.93	2786.5	0	98.8			

											(21.54-41.19)	9					
Continent															0.005	0.176 (0.053 to 0.300)	3.13
	Antarctica	3	429	68	11.73 (0.00-41.07)	100.77	<0.01	98.0									
			Asia						5	195	37	18.06 (1.97-45.17)	62.53	<0.01	93.6		
			Europe						27	3922	807	15.02 (9.41-21.67)	624.79	<0.01	95.8		
			North America						40	9243	2756	29.92 (21.77-38.77)	3023.94	0	98.7		
			Oceania						5	351	56	17.73 (1.13-47.72)	147.22	<0.01	97.3		
			South America						5	432	94	12.92 (0.00-53.38)	316.25	<0.01	98.7		
Mean temperature (°C)															<0.001	-0.251(-0.3810 to -0.1211)	12.12
			<0						25	4098	536	9.68 (5.38-14.97)	561.18	<0.01	95.7		
			0-10						25	2843	677	22.16 (13.07-32.70)	829.54	<0.01	97.1		

	10-15	21	1930	654	33.48 (19.49-48.96)	750.38	<0.01	97.3			
	15-20	18	5103	1766	31.76 (19.41-45.49)	1461.90	<0.01	98.8			
	>20	8	562	171	36.28 (6.36-73.61)	532.74	<0.01	98.7			
Precipitation (mm)									0.085	-0.120(-0.2570 to 0.0164)	4.17
	<200	7	1507	408	20.06 (7.67-36.28)	252.08	<0.01	97.6			
	200-400	24	4571	1476	24.87 (14.02-37.44)	1558.14	<0.01	98.5			
	400-800	25	3692	617	15.14 (8.22-23.48)	816.31	<0.01	97.1			
	>800	44	5042	1341	26.92 (18.20-36.59)	2149.76	0	98.0			

Abrreviations: CI = Confidence interval; x^2 = heterogeneity statistic. p-value < 0.05 is statistically significant; I^2 = heterogeneity index; R^2: proportion of between-study variance explained.

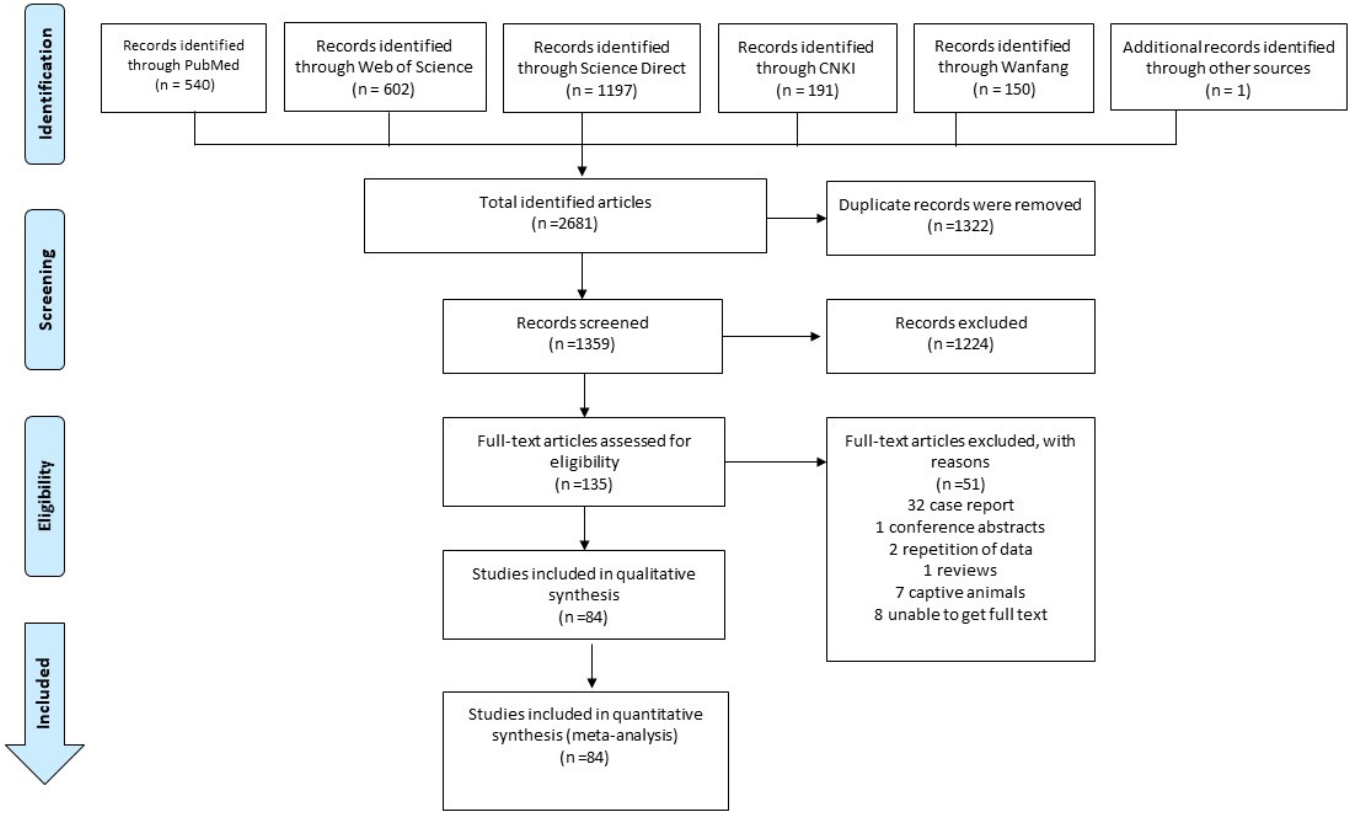

Figure 1. PRISMA flow chart shows the steps used for article screening and selection.

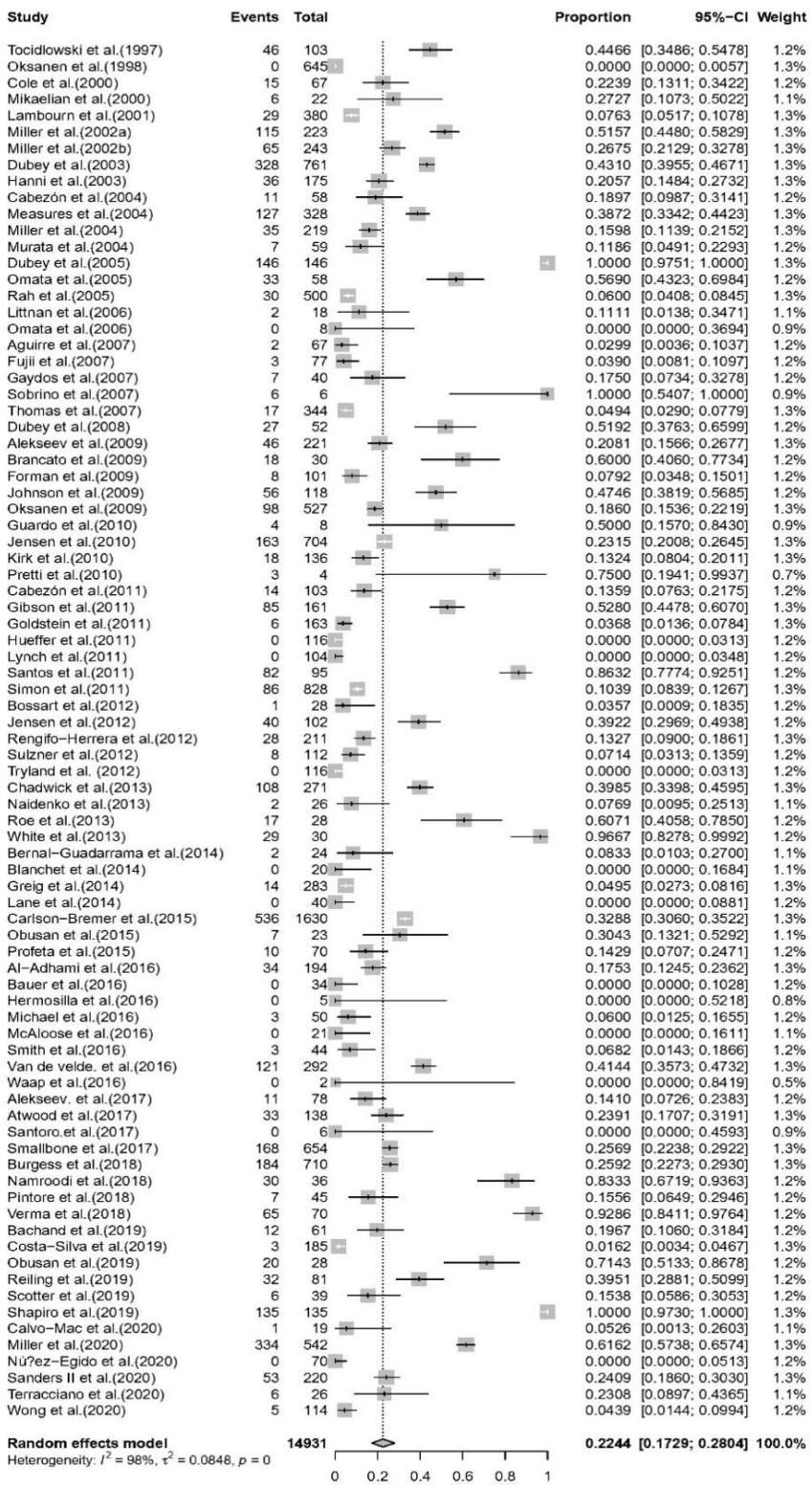

Figure 2. Forest plot of *T. gondii* infection in wild marine mammals. Gray squares and their corresponding lines are the point estimates and 95% CI. The diamond represents the pooled estimate (width denotes 95% CI). Heterogeneity was considered high (I^2 98%)

Th

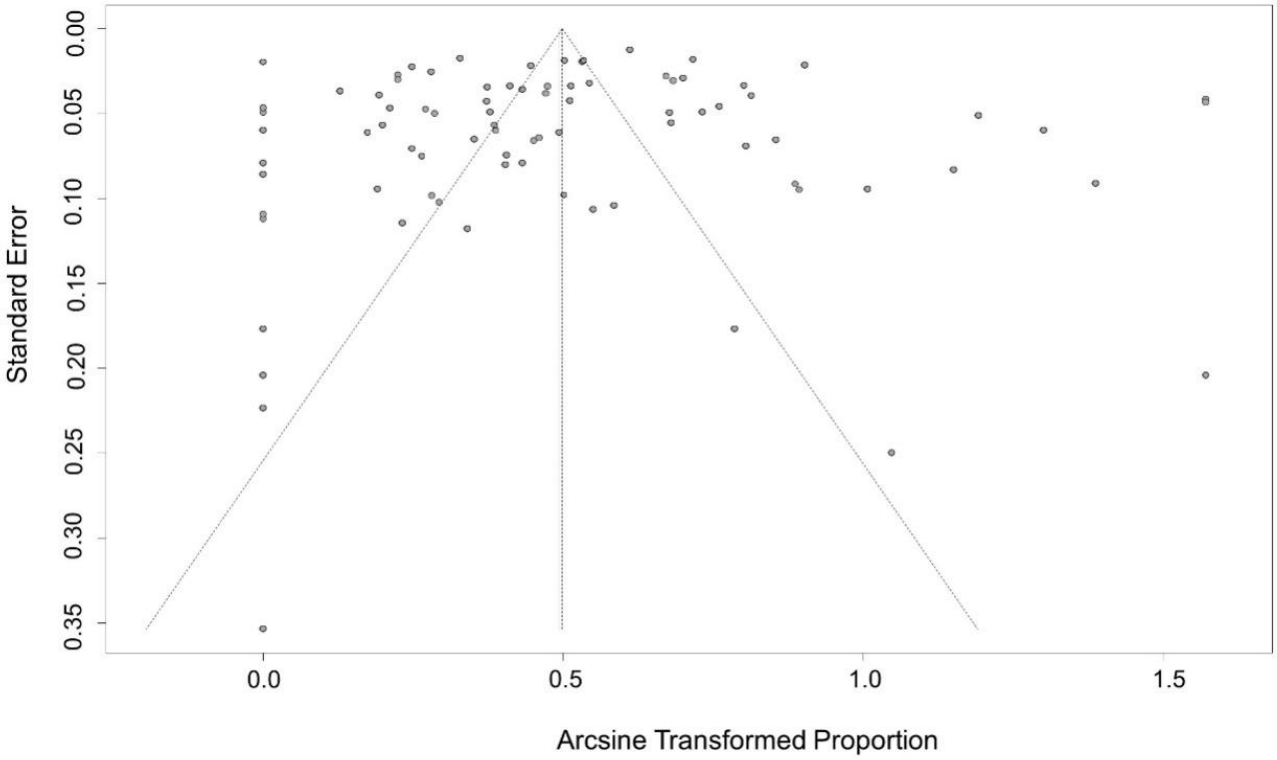

Figure 3. Funnel plot for examination of the publication bias. Each dot represents one study.

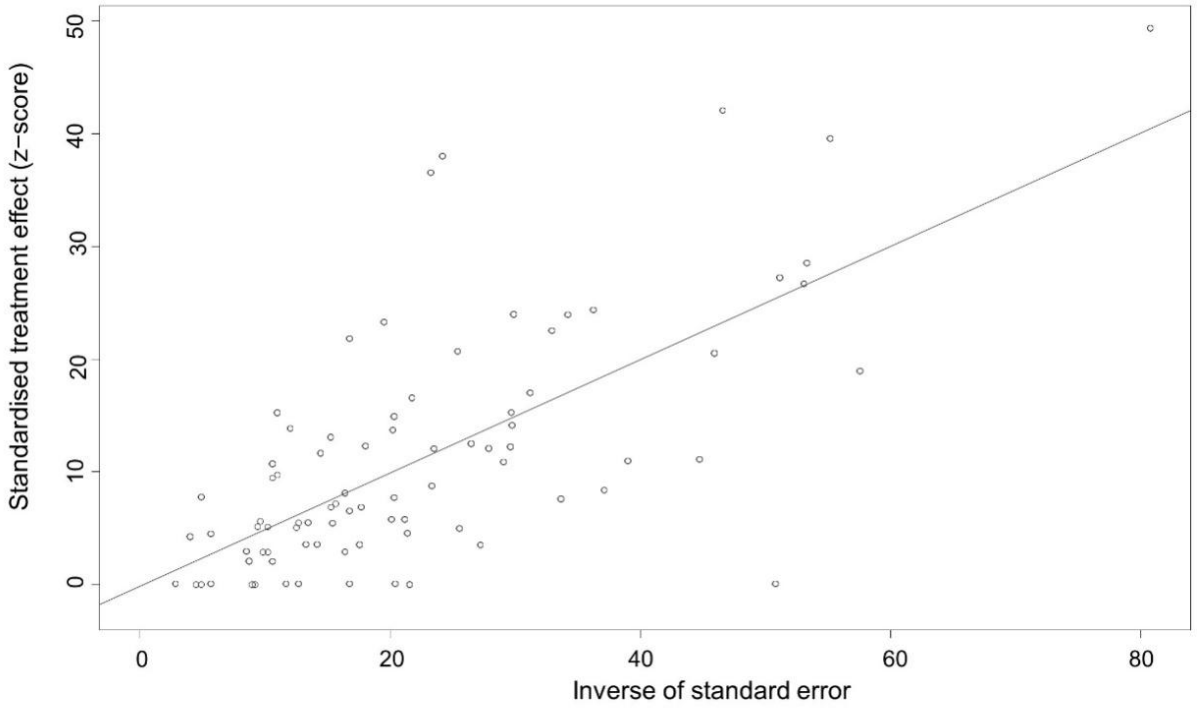

Figure 4. Egger's test for testing the publication bias.

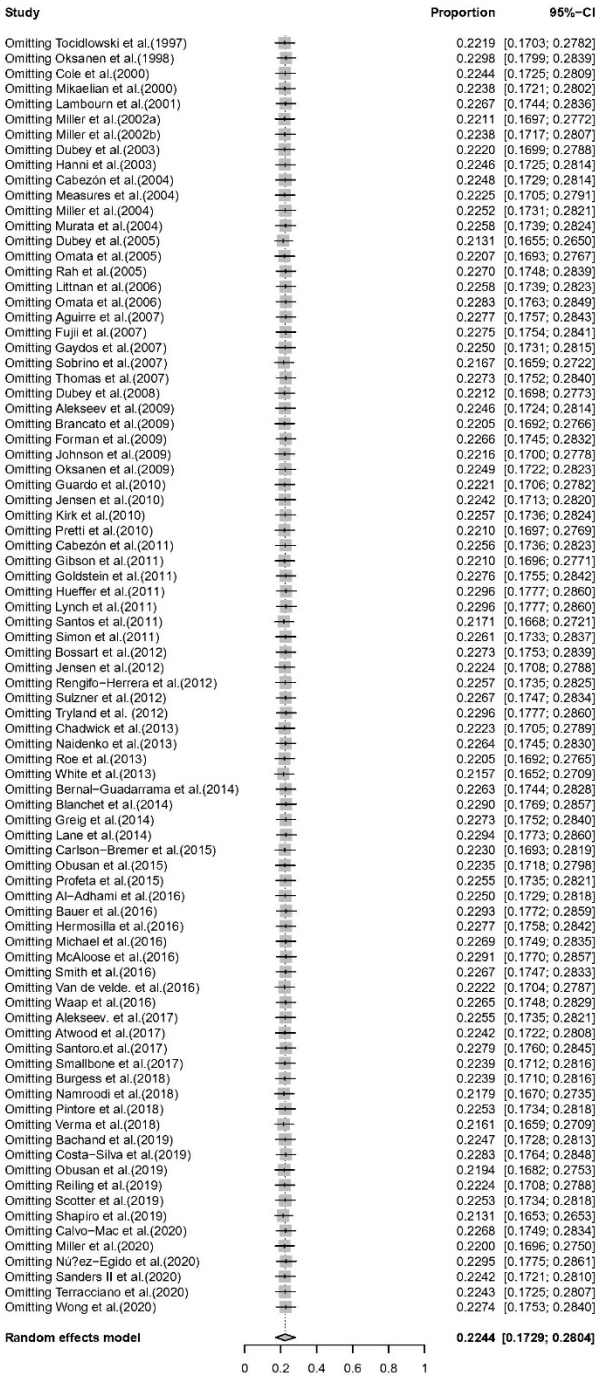

Figure 5. Sensitivity analysis.After removing one study at a time, the remaning studies were recombined using a random-effects model to verify the impact of a single study on the overall results

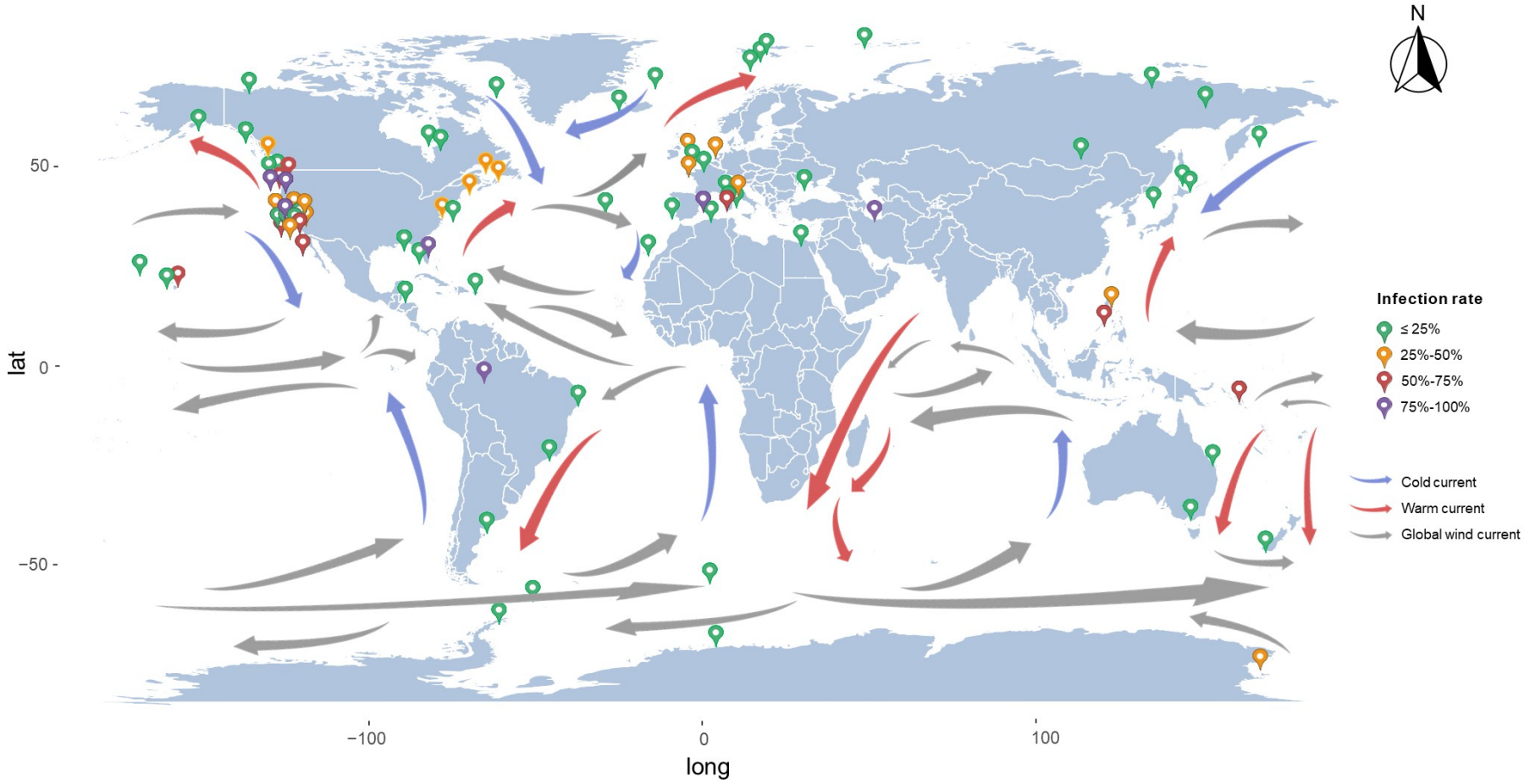

Figure 6. Global map shows the distribution of *T. gondii* infection in wild marine mammals and the direction of surface ocean currents. The ocean circulation may play a role in the dispersion and distribution pattern of *T. gondii* oocysts after entering the sea.

Table S1. PRISMA Checklist for meta-analysis.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Global burden of *Toxoplasma gondii* infection in wild marine mammals: A potential threat to marine ecosystem and public health	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3-4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3-4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5-8
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5-6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5-6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5, Table S2

Section/topic	#	Checklist item	Reported on page #
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5-6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6-8
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7-8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7-8
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7-8
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7-8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7-8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8, Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8, Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	8-9, Figure 3, Figure 4

Section/topic	#	Checklist item	Reported on page #
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	9, Figure 2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9-10, Figure 2
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	8-9, Figure 3, Figure 4
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	9-10, Figure 5, Table 3, Table 4, Table 5
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10-11
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11-17
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	17-18
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data), role of founders for the systematic review.	18

Table S2. Retrieval formulas in database.

database	Search Details
PubMed	
	(((((((("Dugong"[MeSH]) OR* (Dugongs)) OR (Sea Cows)) OR (Cow, Sea)) OR (Cows, Sea)) OR (Sea Cow)) OR (*Dugong dugong*)) OR (*Dugong dugon*))) AND* ((((((*Toxoplasma*"[MeSH]) OR (*Toxoplasma gondii*))
	((("Whales"[MeSH]) OR (Whale)) OR (Giant Bottle-Nosed Whales)) OR (Bottle-Nosed Whale, Giant)) OR (Bottle-Nosed Whales, Giant)) OR (Giant Bottle Nosed Whales)) OR (Giant Bottle-Nosed Whale)) OR (*Berardius*)) OR (Goose-Beaked Whale)) OR (Goose Beaked Whale)) OR (Goose-Beaked Whales)) OR (*Ziphius*)) OR (Beaked Whales)) OR (Beaked Whale)) OR (*Ziphiidae*)) OR (Gray Whale)) OR (Gray Whales)) OR (*Eschrichtius robustus*)) OR (Grey Whale)) OR (Grey Whales)) OR (Whale, Grey)) OR (Whales, Grey)) OR (Right Whale, Southern)) OR (Right Whales, Southern)) OR (Southern Right Whale)) OR (Southern Right Whales)) OR (Whale, Southern Right)) OR (Whales, Southern Right)) OR (*Eubalaena australis*)) OR (Dwarf Sperm Whale)) OR (Dwarf Sperm Whales)) OR (Sperm Whale, Dwarf)) OR (Sperm Whales, Dwarf)) OR (Narwhals)) OR (Narwhal)) OR (*Monodon monoceros*)) OR (Pygmy Right Whale)) OR (Pygmy Right Whales)) OR (Right Whale, Pygmy)) OR (Right Whales, Pygmy)) OR (*Caperea*)) OR (Pygmy Sperm Whale)) OR (Pygmy Sperm Whales)) OR (Sperm Whale, Pygmy)) OR (Sperm Whales, Pygmy)) OR (Right Whale, North Atlantic)) OR (North Atlantic Right Whale)) OR (*Mesoplodon*))) AND ((((((*Toxoplasma*"[MeSH]) OR (*Toxoplasma gondii*)))
	((((((((((((((((((((((((((("Seals, Earless"[MeSH]) OR (Earless Seal)) OR (Earless Seals)) OR (Seal, Earless)) OR (*Phocidae*)) OR (Seals, True)) OR (Seal, True)) OR (True Seal)) OR (True Seals)) OR (Seal, Gray)) OR (Gray Seal)) OR (Gray Seals)) OR (Seals, Gray)) OR (*Halichoerus grypus*)) OR (Seal, Harp)) OR (Harp Seal)) OR (Harp Seals)) OR (Seals, Harp)) OR (*Phoca groenlandica*)) OR (*Pagophilus groenlandicus*)) OR (Seal, Elephant)) OR (Elephant Seal)) OR (Elephant Seals)) OR (Seals, Elephant)) OR (*Mirounga*))) AND ((((((*Toxoplasma*"[MeSH]) OR (*Toxoplasma gondii*)))
	((((((((((((("Sea Lions"[MeSH]) OR (Lion, Sea)) OR (Lions, Sea)) OR (Sea Lion)) OR

	(Sealions)) OR (Sealion)) OR (California Sea Lion)) OR (Lion, California Sea)) OR (*Zalophus californianus*)) OR (Sea Lion, California)) OR (California Sea Lions)) OR (Lions, California Sea)) OR (Sea Lions, California)) OR (*Zalophus*))) AND ((((((*Toxoplasma*"[MeSH]) OR (*Toxoplasma gondii*)))
	((((((((((((((("Porpoises"[MeSH]) OR (Porpoise)) OR (*Phocoenidae*)) OR (Spectacled Porpoise)) OR (Porpoise, Spectacled)) OR (Porpoises, Spectacled)) OR (Spectacled Porpoises)) OR (*Australophocaena dioptrica*)) OR (Finless Porpoise)) OR (Finless Porpoises)) OR (Porpoise, Finless)) OR (Porpoises, Finless)) OR (*Neophocaena phocaenoides*)) OR (Dall Porpoise)) OR (Porpoise, Dall)) OR (*Phocoenoides dalli*))) AND ((((((*Toxoplasma*"[MeSH]) OR (*Toxoplasma gondii*)))
	((((((((((((((((((((("Otters"[MeSH]) OR (Otter)) OR (Giant Otters)) OR (Giant Otter)) OR (Otter, Giant)) OR (Otters, Giant)) OR (*Lontra*)) OR (*Lutra*)) OR (*Pteronura*)) OR (Sea Otters)) OR (Otter, Sea)) OR (Otters, Sea)) OR (Sea Otter)) OR (*Enhydra lutris*)) OR (Clawless Otters)) OR (Clawless Otter)) OR (Otter, Clawless)) OR (Otters, Clawless)) OR (*Aonyx*)) OR (*Lontra canadensis*)) OR (North American River Otter))) AND ((((((*Toxoplasma*"[MeSH]) OR ((*Toxoplasma gondii*)))
	((((*Trichechus*"[MeSH]) OR (Manatees)) OR (Manatee))) AND ((((((*Toxoplasma*"[MeSH]) OR (*Toxoplasma gondii*)))
	((("Dolphins"[MeSH]) OR (Dolphin)) OR (Whale, Melon-Headed)) OR (*Peponocephala electra*)) OR (Many-Toothed Blackfish)) OR (Many Toothed Blackfish)) OR (Melon-Headed Whale)) OR (Melon Headed Whale)) OR (Melon-Headed Whales)) OR (Whale, Pygmy Killer)) OR (*Feresa attenuata*)) OR (Pygmy Killer Whale)) OR (Pygmy Killer Whales)) OR (Susus)) OR (*Platanista*)) OR (*Platanistas*)) OR (White-Beaked Dolphins)) OR (White Beaked Dolphins)) OR (White-Beaked Dolphin)) OR (*Lagenorhynchus albirostris*)) OR (*Lagenorhynchus albirostri*)) OR (Amazon Dolphins)) OR (*Inia geoffrensis*)) OR (Amazon River Dolphins)) OR (*Langenorhynchus obliquidens*)) OR (*Langenorhynchus obliquiden*)) OR (Pacific White-Sided Dolphins)) OR (Pacific White Sided Dolphins)) OR (Pacific White-Sided Dolphin)) OR (Chinese River Dolphin)) OR (*Lipotes vexillifer*)) OR (Yangtze River Dolphin)) OR (Baiji)) OR (*Baijus*)) OR

	(Atlantic White-Sided Dolphins)) OR (Atlantic White Sided Dolphins)) OR (Atlantic White-Sided Dolphin)) OR (*Lagenorhynchus acutus*)) OR (Fraser's Dolphin)) OR (Dolphin, Fraser's)) OR (Fraser Dolphin)) OR (Frasers Dolphin)) OR (*Lagenodelphis hosei*)) OR (*Lagenodelphis hoseus*)) OR (Ganges Dolphin)) OR (*Planista gangetica*)) OR (*Planista gangeticas*)) OR (Ganges River Dolphin)) OR (Irrawaddy River Dolphin)) OR (*Orcaella brevirostris*)) OR (*Orcaella brevirostri*)) OR (La Plata Dolphin)) OR (*Pontoporia blainvillei*)) OR (*Pontoporia blainvilleus*)) OR (*Franciscana*)) OR (*Franciscanas*)) OR (*Lagenorhynchus*)) OR (Ocean Dolphins)) OR (Ocean Dolphin)) OR (Marine Dolphins)) OR (Marine Dolphin)) OR (Delphinidae)) OR (Piebald Dolphins)) OR (Piebald Dolphin)) OR (*Cephalorhynchus*)) OR (Right Whale Dolphins)) OR (Right Whale Dolphin)) OR (*Lissodelphis*)) OR (*Lissodelphi*)) OR (Risso's Dolphin)) OR (Risso Dolphin)) OR (Rissos Dolphin)) OR (*Grampus griseus*)) OR (Gray Grampus)) OR (Rough-Toothed Dolphin)) OR (Rough Toothed Dolphin)) OR (Rough-Toothed Dolphins)) OR (*Steno bredanensis*)) OR (Sousa)) OR (Humpback Dolphin)) OR (Humpback Dolphins)) OR (Tucuxi Dolphin)) OR (Tucuxi Dolphins)) OR (*Sotalia fluviatilis*)) OR (*Sotalia fluviatili*)) OR (Whale, False Killer)) OR (*Pseudorca crassidens*)) OR (*Pseudorca crassiden*)) OR (False Killer Whale)) OR (False Killer Whales))) AND ((((((*Toxoplasma*"[MeSH]) OR (*Toxoplasma gondii*)))
	((marine mammals)) AND ((((((*Toxoplasma*"[MeSH]) OR (*Toxoplasma gondii*)))
	((polar bear)) AND ((((((*Toxoplasma*"[MeSH]) (*Toxoplasma gondii*)))
Web of Science	
#1*	TS*=marine mammals
#2	TS=(Dolphins OR Dolphin OR Whale, Melon-Headed OR *Peponocephala electra* OR Many-Toothed Blackfish OR Many Toothed Blackfish OR Melon-Headed Whale OR Melon Headed Whale OR Melon-Headed Whales OR Whale, Pygmy Killer OR *Feresa attenuata* OR Pygmy Killer Whale OR Pygmy Killer Whales OR Susus OR *Platanista* OR *Platanistas* OR White-Beaked Dolphins OR White Beaked Dolphins OR White-Beaked Dolphin OR *Lagenorhynchus albirostris* OR *Lagenorhynchus albirostri* OR Amazon Dolphins OR *Inia geoffrensis* OR Amazon River Dolphins OR *Langenorhynchus obliquidens* OR *Langenorhynchus obliquiden* OR

	Pacific White-Sided Dolphins OR Pacific White Sided Dolphins OR Pacific White-Sided Dolphin OR Chinese River Dolphin OR *Lipotes vexillifer* OR Yangtze River Dolphin OR Baiji OR *Baijus* OR Atlantic White-Sided Dolphins OR Atlantic White Sided Dolphins OR Atlantic White-Sided Dolphin OR *Lagenorhynchus acutus* OR Fraser's Dolphin OR Dolphin, Fraser's OR Fraser Dolphin OR Frasers Dolphin OR *Lagenodelphis hosei* OR *Lagenodelphis hoseus* OR Ganges Dolphin OR *Planista gangetica* OR *Planista gangeticas* OR Ganges River Dolphin OR Irrawaddy River Dolphin OR *Orcaella brevirostris* OR *Orcaella brevirostri* OR La Plata Dolphin OR *Pontoporia blainvillei* OR *Pontoporia blainvilleus* OR *Franciscana* OR *Franciscanas* OR *Lagenorhynchus* OR Ocean Dolphins OR Ocean Dolphin OR Marine Dolphins OR Marine Dolphin OR Delphinidae OR Piebald Dolphins OR Piebald Dolphin OR *Cephalorhynchus* OR Right Whale Dolphins OR Right Whale Dolphin OR *Lissodelphis* OR *Lissodelphi* OR Risso's Dolphin OR Risso Dolphin OR Rissos Dolphin OR Grampus griseus OR Gray Grampus OR Rough-Toothed Dolphin OR Rough Toothed Dolphin OR Rough-Toothed Dolphins OR *Steno bredanensis* OR Sousa OR Humpback Dolphin OR Humpback Dolphins OR Tucuxi Dolphin OR Tucuxi Dolphins OR *Sotalia fluviatilis* OR *Sotalia fluviatili* OR Whale, False Killer OR *Pseudorca crassidens* OR *Pseudorca crassiden* OR False Killer Whale OR False Killer Whales)
#3	TS= (*Trichechus* OR Manatees OR Manatee)
#4	TS= (Otters OR Otter OR Giant Otters OR Giant Otter OR Otter, Giant OR Otters, Giant OR *Lontra* OR *Lutra* OR *Pteronura* OR Sea Otters OR Otter, Sea OR Otters, Sea OR Sea Otter OR *Enhydra lutris* OR Clawless Otters OR Clawless Otter OR Otter, Clawless OR Otters, Clawless OR *Aonyx* OR *Lontra canadensis* OR North American River Otter)
#5	TS= (Porpoises OR Porpoise OR *Phocoenidae* OR Spectacled Porpoise OR Porpoise, Spectacled OR Porpoises, Spectacled OR Spectacled Porpoises OR *Australophocaena dioptrica* OR Finless Porpoise OR Finless Porpoises OR Porpoise, Finless OR Porpoises, Finless OR *Neophocaena phocaenoides* OR Dall Porpoise OR Porpoise, Dall OR *Phocoenoides dalli*)
#6	TS= (Sea Lions OR Lion, Sea OR Lions, Sea OR Sea Lion OR Sealions OR Sealion OR California Sea Lion OR Lion, California Sea OR *Zalophus californianus* OR Sea Lion, California OR California Sea Lions OR Lions, California Sea OR Sea Lions, California OR Zalophus)
#7	TS= (Seals, Earless OR Earless Seal OR Earless Seals OR Seal, Earless OR *Phocidae* OR Seals,

	True OR Seal, True OR True Seal OR True Seals OR Seal, Gray OR Gray Seal OR Gray Seals OR Seals, Gray OR *Halichoerus grypus* OR Seal, Harp OR Harp Seal OR Harp Seals OR Seals, Harp OR *Phoca groenlandica* OR *Pagophilus groenlandicus* OR Seal, Elephant OR Elephant Seal OR Elephant Seals OR Seals, Elephant OR *Mirounga*)
#8	TS= (Whales OR Whale OR Giant Bottle-Nosed Whales OR Bottle-Nosed Whale, Giant OR Bottle-Nosed Whales, Giant OR Giant Bottle Nosed Whales OR Giant Bottle-Nosed Whale OR *Berardius* OR Goose-Beaked Whale OR Goose Beaked Whale OR Goose-Beaked Whales OR *Ziphius* OR Beaked Whales Beaked Whale OR *Ziphiidae* OR Gray Whale OR Gray Whales OR *Eschrichtius robustus* OR Grey Whale OR Grey Whales OR Whale, Grey OR Whales, Grey OR Right Whale, Southern OR Right Whales, Southern OR Southern Right Whale OR Southern Right Whales OR Whale, Southern Right OR Whales, Southern Right OR *Eubalaena australis* OR Dwarf Sperm Whale OR Dwarf Sperm Whales OR Sperm Whale, Dwarf OR Sperm Whales, Dwarf OR Narwhals OR Narwhal OR *Monodon monoceros* OR Pygmy Right Whale OR Pygmy Right Whales OR Right Whale, Pygmy OR Right Whales, Pygmy OR *Caperea* OR Pygmy Sperm Whale OR Pygmy Sperm Whales OR Sperm Whale, Pygmy OR Sperm Whales, Pygmy OR Right Whale, North Atlantic OR North Atlantic Right Whale OR *Mesoplodon*)
#9	TS= (Dugong OR Dugongs OR Sea Cows OR Cow, Sea OR Cows, Sea OR Sea Cow OR *Dugong dugong* OR *Dugong dugon*)
#10	TS= polar bear
#11	TS= (*Toxoplasma* OR *Toxoplasma*s OR *Toxoplasma gondii* OR *Toxoplasma gondius* OR *gondius, Toxoplasma*)
#12	#11 AND #1
#13	#11 AND #2
#14	#11 AND #3
#15	#11 AND #4
#16	#11 AND #5
#17	#11 AND #6
#18	#11 AND #7

#19	#11 AND #8
#20	#11 AND #9
#21	#11 AND #10
Science Direct	
	Marine mammals AND *Toxoplasma*
	Dolphins AND *Toxoplasma*
	Trichechus AND *Toxoplasma*
	Otters AND *Toxoplasma*
	Sea Lions AND *Toxoplasma*
	Porpoises AND *Toxoplasma*
	Seal AND *Toxoplasma*
	Whales AND *Toxoplasma*
	Whales AND *Toxoplasma*
	Polar bear AND *Toxoplasma*
CNKI	
	(Theme: marine mammals) AND (theme: Dolphins) AND (theme: *Trichechus*) AND (theme: Otters) AND (theme: Sea Lions) AND (theme: Earless Seal) AND (theme: Whales) AND (theme: Whales) AND (theme: polar bear) AND (theme: *Toxoplasma*)
Wanfang	
	Theme:(marine mammals or Dolphins or *Trichechus* or Otters or Porpoises or Sea Lions or Earless Seal or Whales or Dugong) and theme:(*Toxoplasma*)

OR*: Boolean operator, the retrieval form written as A OR B indicates that all the records in the database with the retrieval words A or B, or both A and B are hit records.

AND*: Boolean operator, the retrieval form written as A AND B indicates that only the records with both the retrieval words A and B in the database are the hit records.

#*: Steps of retrieval.

TS*: Field Tags represents “Topic” in Web of Science.

Table S3. R code for meta-analysis.

No transformation (PRAW)	rate<-transform (m1, r= event/n); shapiro.test (rate$p)
Logarithmic conversion (PLN)	rate<-transform (m1, log=log(event/n)); shapiro.test (rate$log)
Logit transformation (PLOGIT)	rate<-transform (m1, logit=log((event/n)/(1-event/n))); shapiro.test (rate$logit)
Arcsine transformation (PAS)	rate<-transform (m1, arcsin.size=asin(sqrt(event/(n+1)))); shapiro.test (rate$arcsin)
Double-arcsine transformation (PFT)	rate<-transform (m1, darcsin=0.5*(asin(sqrt(event/(n+1))) +asin((sqrt(event+1)/(n+1))))); shapiro.test (rate$ darcsin)
Meta analysis	metarate <- metaprop (event, n, study, data = rate, sm="PAS", incr=0.5, allincr=TRUE, addincr=FALSE, title="")
Forest plots	forest (metarate,digits=4,xlim=c(0,1), comb.fixed = F)
Funnel chart	funnel (metarate)
Egger's test	metabias (metarate, method="linreg", plotit=TRUE, k.min=10)
The sensitivity analysis	metainf (metarate, pooled = "random") forest (metainf (metarate, pooled = "random"),digits=4)
Subgroup analysis	meta1<- metaprop (event, n, study, data = subgroup rate, sm="PFT", incr=0.5, allincr=TRUE, addincr=FALSE, title=" ", byvar= subgroup title, print.byvar=TRUE) forest (meta1, digits=4, xlim=c (0, 1))
Meta-regression analysis	metareg (meta1, ~covariate title)

Table S4. Normal distribution test for the normal rate and the different transformation of the normal rate.

Transformation form	*W*	*P*
PRAW	0.84041	4.462e-08
PLN	NaN	NA
PLOGIT	NaN	NA
PAS	0.93541	0.0003898
PFT	0.92356	9.574e-05

PRAW: untransformed, original rate; PLN: logarithmic transformation; PLOGIT: logit transformation; “PAS”: arcsine transformation; PFT: double arcsine transformation; NaN: meaningless number; NA: missing data.

Table S5. Full citation of the included studies in choronological order.

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