Prevalence of *Toxoplasma gondii* in pet dogs in mainland China: A meta-analysis

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Abstract

Toxoplasmosis, caused by *Toxoplasma gondii*, is one of the most common zoonosis in the world, and can cause severe adverse consequences in pregnant women and immunosuppressed people. Throughout the world dogs are popular companion animals, however they are also important intermediate host of *T. gondii* and may play an instrumental role in mechanically transmitting *Toxoplasma* infection to humans. Therefore, an assessment of the level of *T. gondii* infection in pet dogs is of public health importance. To estimate the overall serological prevalence of toxoplasmosis in pet dogs in mainland China, we performed a meta-analysis using five bibliographic databases: CNKI, WanFang, VIP Chinese Journal Databases, PubMed and Google Scholar. A total of 60 articles, including data on 78719 pet dogs, published between 1998 and 2016 fulfilled the final eligibility criteria. The overall seroprevalence of *T. gondii* infection in pet dogs was 11.1% (95% CI: 10.1% to 12.1%). The seroprevalence of toxoplasmosis in >one-year-old dogs was 1.59 times more than that in ≤one-year-old group (RR=1.59, 95%CI=1.15, 2.21). There was no significant difference observed in pooled seroprevalence between male and female dogs (RR=1.02, 95%CI=0.91, 1.16). In terms of geographical regions, the lowest seroprevalence of toxoplasmosis infection (5.8%) was in North China and the highest (16.8%) was in Southwest of China. Results from this study showed that toxoplasmosis is common in pet dogs in mainland China, indicating that control measures by owners may reduce human exposure to *T. gondii* via dog contacts.

Key words: *Toxoplasma gondii*, pet dogs, mainland China, meta analysis

Introduction

Toxoplasmosis, caused by the obligate, intracellular protozoan *Toxoplasma gondii*, is one of the most common zoonosis in the world. *Toxoplasma gondii* is able to infect almost all warm-blooded animals, including humans and domesticated and wild animals (Dubey et al., 2011; Hill and Dubey, 2013; Zhang et al., 2015a). The wide distribution of *T. gondii* may be attributed to complex transmission patterns and the co-evolution of the parasite and hosts (Berday et al., 1995; Lamberton et al., 2008). Felids serve as its only definitive hosts and discharge oocysts in their feces (Dabritz and Conrad, 2010; Dubey et al., 2012b). Humans and other animals may be infected with *T. gondii* through ingestion of these oocysts directly or through eating undercooked or raw meat which contains tissue cysts (Dubey, 2004; Montoya and Liesenfeld, 2004; Torda, 2001; Zhao et al., 2011). Toxoplasmosis is often benign, or associated with mild non-specific clinical symptoms in most humans, however epidemiological, serological and laboratory studies indicate associations between *T. gondii* infection and schizophrenia (Dickerson et al., 2014; Torrey et al., 2012; Wang et al., 2013; Webster, 2001). In addition toxoplasmosis in congenitally infected children and immunosuppressed patients such as those with AIDS and organ transplant recipients can result in severe adverse consequences, including mortality (Dubey et al., 2012a; Montoya and Liesenfeld, 2004).

Dogs are an important intermediate host of *T. gondii*, and although in general they, like humans, are dead end hosts, *T. gondii* oocysts can traverse the intestinal tract of dogs and be excreted in the feces (Lindsay et al., 1997). In addition, mechanical transmission of *T. gondii* oocysts, from dogs via their body surfaces, mouth parts or feet, is a potential risk factor for *T. gondii* infection in humans (Etheredge et al., 2004; Frenkel et al., 1995), particularly from pet dogs due to their coprophagous habits, rolling behaviors, and high contact rates with their owners (Dubey et al., 2009; Frenkel and Parker, 1996; Lindsay et al., 1997; Mwambe et al., 2013).

With economic development and great societal change over time (i.e. the increasing proportion of older people), particularly in mainland China, the number of pet dogs is rapidly

increasing. In 2003, the number of breeding dogs in China was estimated to be more than 200 million (Ding and Xia, 2005); with this number increasing at an annual rate of ~20% (Zhang and Xu, 2003). An observation in Minhang district of Shanghai reported that the number of pet dogs in 2001 was 2543, but that it had increased by 3.5 times to 8912 in 2006 (Zhang et al., 2008). In 2005, the Health Ministry of China showed that the average infection rate of *T. gondii* in the general human population was 7.88%, while the rate in pet breeders was generally higher (Xu et al., 2005). A similar finding was reported by Kortbeek and his colleagues in the Netherlands (Kortbeek et al., 2004). Further research demonstrated that among infected people, 52% of infections could be attributed to the activity of raising a dog (Fu et al., 2004). Dogs, which are exposed to *T. gondii* oocysts and mechanically transport them and potentially expose humans, are also more likely to be infected themselves. Therefore dogs could also serve as sentinels for humans, with the infection prevalence in pet dogs potentially linked to environmental contamination with oocysts. Therefore, as pet dog populations increase, a study on *T. gondii* infection in pet dogs is timely and will have practical implications for public health practitioners. To our knowledge, there is no exhaustive documented data about the prevalence of *T. gondii* infection in pet dogs in mainland China, therefore this meta-analysis was carried out to estimate the seroprevalence of toxoplasmosis in such animals across the country. Potential influential factors such as dog's age, gender or geographical location on *T. gondii* infection were investigated.

Materials and methods

Search strategy

A literature search for publications up to July 7, 2016 regarding *T. gondii* infection in pet dogs in mainland China was performed using five bibliographic databases: China National Knowledge Infrastructure (CNKI), WanFang, VIP Chinese Journal Databases, PubMed and Google Scholar. Pet dogs refer to dogs that people feed and use as a companion, often to eliminate loneliness or for recreational purposes. In this paper, we assumed that all domesticated dogs were pet dogs and we excluded countryside dogs and working dogs. However, it must be noted that we also excluded data on Tibetan Mastiffs and Pit Bulls, as they are banned from being raised in most parts of China. We used search terms “pet dogs”,

“*Toxoplasma*”, and “China” in the English databases and “chongwu”, “gou”, and “gongxingchong” in the Chinese databases. No restrictions were imposed. In addition, we reviewed the reference lists of retrieved studies and recent reviews. We did not contact authors of original studies for additional information. No attempt was made to identify unpublished reports.

Study selection

Study selection was based on an initial screen of identified abstracts and/or titles and a second screen of full-text articles. Studies were considered eligible if they meet the following criteria: 1) targeted objects must be pet dogs; 2) locations must be within mainland China; 3) purpose must be to study the seroprevalence of *T. gondii*, and the information on the number of dogs examined and the number of positive dogs reported. Studies were excluded if they did not fulfill all of these criteria.

Data extraction

We extracted data using a standardized data-collection form. Information was recorded as follows: first author, publication year, calendar period of study, geographical region of study, diagnostic tests (i.e. ELISA, IHA, etc), cut-off point (if reported), total number of pet dogs and number of *T. gondii* serologically positive pet dogs. If the article was stratified by the dog’s age and/or gender, we extracted the relevant number at each layer of stratification.

Statistical analysis

Point estimates and their 95% confidence intervals of pooled seroprevalence of all included studies were calculated. Forest plots were used to visualize the results of each study and the heterogeneity among studies. The heterogeneity was expected in advance, and statistical methods with I^2 and Cochrane Q statistics were used to quantify the variations. Simultaneously, we conducted a subgroup analysis stratified by dog’s age, gender, geographic regions or tests used to assess the impacts of these factors on *Toxoplasma* infection in pet dogs. Publication bias of studies was statistically examined with the Begger test in Stata/SE (version 12.0). We also conducted a sensitivity analysis to investigate the influence of a single

study on the overall risk estimate by omitting one study in turn. A p value of <0.05 was considered statistically significant, except where otherwise specified.

We performed and reported this meta-analysis according to the PRISMA guideline (Moher et al., 2009), and the PRISMA checklist for the information items in this analysis are reported in Additional file 1.

Results

Literature search

A total of 60 publications were suitable for quantitative analysis (Fig. 1). The included studies were published between 1998 and 2015. The total number of pet dogs was 78,719, with a range of 52 to 28,783 in each study. The seroprevalence of toxoplasmosis in pet dogs was reported as being between 0 and 34.9% among the included studies. The references for the included studies and their detailed characteristics are listed in Additional file 2.

Main analysis

As displayed in Table 1, there was a wide variation in the prevalence estimates among different studies. Both the statistics Q and I^2 indicated that a random-effects model should be applied in this analysis. The pooled prevalence of *T. gondii* infection in pet dogs was 11.1% (95% CI: 10.1% to 12.1%). We also performed the subgroup analyses stratified by dog's age, gender, geographic location, or test used. As there was a significantly high level of heterogeneity among studies within each subgroup (all $P < 0.01$), all estimates of the pooled prevalence for each subgroup were calculated with a random-effects model. Of 60 studies, 16 studies investigated *Toxoplasma* infection in pet dogs by age. The seroprevalence of toxoplasmosis in pet dogs was significantly associated with age, as the infection prevalence in >one-year-old dogs was 1.59 times more than that in ≤one-year-old dogs (RR=1.59, 95%CI=1.15, 2.21). A total of 18 studies provided information on gender, but no significant difference was observed in the estimate between male and female dogs. On the basis of geographical regions, the lowest seroprevalence of toxoplasmosis infection (5.8%) was in North China and the highest (16.8%) was in Southwest of China. A total of 45 studies

employed either ELISA or IHA test, and no significant difference in *T. gondii* seroprevalence across the studies was observed between the two methods.

Sensitivity and publication bias analyses

The sensitivity analyses showed that the pooled prevalence, after omitting one study at a time, was not substantially influenced by any single study (see Additional file 3). Egger's test indicated no evidence of publication bias ($t=-1.79$, $P>0.05$); whereas Begg's regression test showed publication bias in favor of a positive infection rate ($Z=2.56$, $P<0.05$) (see Additional file 4). However, we then used the trim and fill method (Wang et al., 2008) to evaluate the impact of publication bias on our final results with little publication bias effect observed, supporting the authenticity of our results (Fig. 2).

Discussion

As dogs, important intermediate hosts of *T. gondii*, are one of the most popular companion animals to humans, an assessment of the level of *T. gondii* infection in pet dogs is of importance to public health. This is due to the risk of mechanical (Dubey et al., 2009; Etheredge et al., 2004; Frenkel et al., 1995; Frenkel and Parker, 1996; Lindsay et al., 1997; Mwambe et al., 2013) or potentially direct from feces (Lindsay et al., 1997), transmission of *T. gondii* to humans. In this study, the data from 78719 pet dogs with 5187 positive *T. gondii* cases, from 60 articles were evaluated. The seroprevalence of *T. gondii* among pet dogs in mainland China, from 1998 to 2016, was 11.1% with the 95% confidence interval of 10.1% to 12.1%. There was no significant difference between infection levels in pet dogs (11.1%) when compared with the infection levels in stray dogs (14.05%) in seven cities of Northern China (Zhang et al., 2015b), indicating similar exposure levels and limited or no infection/disease control in pet dogs.

We conducted subgroup analyses by pet dogs' age, gender, regions or main serological tests. The results showed a significant positive relationship between pet dog age and *T. gondii* seroprevalence. Indeed, as a dog ages, the chance of exposure to *T. gondii* in the surrounding environment and the risk of exposure and subsequent acquisition of infection will, not

surprisingly, increase. Once antibodies to *T. gondii* have been produced after exposure to/infection with the parasite, they may persist for several years and may not reflect an active infection, or current exposure, but they are reflective of general parasite levels in the local environment. As observed in stray dogs (Zhang et al., 2015b), no significant difference was shown between male and female dogs, indicating that exposure and risk behaviors do not differ between genders.

The pooled seroprevalence of toxoplasmosis in pet dogs at the level of geographical regions showed the highest infection rate in Southwestern China, which includes Guizhou, Sichuan, Yunnan provinces and Chongqing Municipality. All the four regions are characterized by mild and wet climates with an annual rainfall of 600-1800mm and temperature of 10-22°C. This climate provides favorable conditions for the survival and sporulation of *T. gondii* oocysts in the environment. In contrast, the lowest infection rate is in Northern China, covering Beijing, Hebei, Tianjin, Inner Mongolia, with less rainfall and lower temperatures, highlighting the potential for climatic risk factors to be used in *T. gondii* disease risk mapping to identify potential areas of human exposure risk.

There are at least two limitations in our meta-analysis. First, data extraction was limited to stratification of only dog sex, age, geographical location or serological test, making it impossible to assess the effects of other potential factors, such as pet housing, pet human contact rate, etc. The other is in terms of detection for *T. gondii* infections. Pet dogs with active or previous infections of *T. gondii* can not be distinguished due to the common serological techniques employed. Antigen or parasite DNA based methods can detect current active infections, but may be less sensitive and were not commonly used in the published literature. However, it should be emphasized that this is the first study, to our knowledge, to estimate the overall seroprevalence of *T. gondii* in pet dogs in mainland China. The seroprevalence level in pet dogs could indicate to what degree the environment has been contaminated with oocysts, and therefore the potential infection risk to humans, with our findings indicating a higher infection risk in Southwestern China. Moreover, with pet animals, particularly dogs and/or cats (definitive host for the parasite) becoming increasingly common

(Zhong, 2014; Zhou, 2013), in both urban and rural areas without restrictions, the risk of human exposure to *T. gondii* may increase. People usually walk their pet dogs outside where they can roll in and/or eat soil contaminated with oocysts from cat feces. Humans in mainland China, now regularly play with their pet dogs, including extended periods of physical contact, including allowing them to lick them. Such behaviors and the relatively high seroprevalence of *T. gondii* in pet dogs which we report here may result in increased *T. gondii* infections in pet owners, particularly through mechanical transmission (Frenkel et al., 1995).

Toxoplasma gondii infection is linked with behavioral changes in humans, with substantial evidence suggesting an increased prevalence of *T. gondii* infection in individuals with schizophrenia (Flegr, 2015). As evidence grows for the association between *T. gondii* infection with bipolar disorder, addiction, obsessive–compulsive disorder (Sutterland et al., 2015), and other diseases (Flegr et al., 2014), it is imperative that we understand the infection risks associated with life style behaviors and how these may be increasing with, for example, the increased dog and cat pet ownership in China, and their impact on public health. Future research is needed to qualify and quantify the infection risk from these pet dogs and cats to humans, ideally using epidemiological and parasite population genetic techniques to inform on parasite transmission dynamics between key intermediate and definitive host groups, such as we have performed for another indirectly transmitted zoonotic parasite, *Schistosoma japonicum* (Lu et al., 2010).

Conclusion

Toxoplasmosis is common in pet dogs in mainland China, and the overall seroprevalence differs among dog ages and regions. Due to the severe consequences caused by the parasite in pregnant women, immunosuppressed people, and the links with mental disorders, combined with the increasing number of domestic cats and dogs, control measures and education to reduce human exposure to *T. gondii* via eating undercooked red and poultry meat, but also through dog contact should be set on an agenda in mainland China.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

YG and DL conceived of and designed the study. YG and HD carried out the screen of the literature and data extraction, and were assisted by DL and PHL in analyzing the results. YG, DL and PHL drafted the manuscript. All authors read and approved the final manuscript.

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Figure legends

Fig. 1 Flow diagram of the selection of eligible studies

Fig. 2 Filled funnel plot (with pseudo 95% confidence limits) for bias test

Table 1 Pooled seroprevalence of *T. gondii* in pet dogs by age, gender, geographical location and diagnostic test

Group	No. of studies	Seroprevalence (95% CI)	Q	P-heterogeneity	I ²
Total	60	0.111(0.101, 0.121)	5966.19	<0.001	0.990
Age					
≤1 year	16	0.111(0.072, 0.150)	141.33	<0.001	0.894
>1 year	16	0.172(0.134, 0.209)	131.88	<0.001	0.886
Mixed	37	0.115(0.092, 0.139)	3826.93	<0.001	0.991
Gender					
Male	18	0.160(0.123, 0.197)	139.17	<0.001	0.878
Female	18	0.160(0.120, 0.200)	147.04	<0.001	0.884
Mixed	36	0.113(0.089, 0.137)	3782.72	<0.001	0.991
Regions					
North China	11	0.058(0.043, 0.073)	303.49	<0.001	0.967
Northeast	4	0.146(0.099, 0.194)	14.41	0.002	0.792
East China	18	0.120(0.082, 0.158)	3906.13	<0.001	0.996
South Central China	13	0.124(0.089, 0.159)	289.92	<0.001	0.962
Southwest	6	0.168(0.093, 0.243)	154.50	<0.001	0.968
North-west	7	0.130(0.078, 0.183)	83.44	<0.001	0.928
Method					
ELISA	20	0.134(0.101, 0.166)	339.40	<0.001	0.944
IHA	25	0.104(0.080, 0.127)	784.98	<0.001	0.971

366 Additional file 1. PRISMA checklist
367 Additional file 2. Reference list for included studies and their characteristics
368 Additional file 3. Sensitivity analyses with MetaAnalyst Beta 3.13
369 Additional file 4. Begg's funnel plot (with pseudo 95% confidence limits) for bias test
370

Figure 1

Figure 2