Toxoplasma gondii infection in farmed wild boars (*Sus scrofa*) in three cities of Northeast China

Meng-Jie Bai [1], Yang Zou [1,2], Hany M. Elsheikha[3], Jian-Gang Ma [1,2], Wen-Bin Zheng [1,2], Quan Zhao [2], Xiao-Xuan Zhang [1,2,*], Xing-Quan Zhu[1,4,*]

[1] State Key Laboratory of Veterinary Etiological Biology, Key Laboratory of Veterinary Parasitology of Gansu Province, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu Province 730046, PR China

[2] College of Animal Science and Technology, Jilin Agricultural University, Changchun, Jilin Province 130118, PR China

[3] Faculty of Medicine and Health Sciences, School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK

[4] Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou, Jiangsu Province 225009, PR China

Abstract

The apicomplexan protozoan parasite *Toxoplasma gondii* is a widely distributed etiological agent of food-borne illness. This parasite can cause production losses in livestock and serious disease in humans through consumption of contaminated meat. Pig's meat is the most likely source of human infection and wild boars may play a role in the transmission of *T. gondii* by serving as a reservoir host. This study aimed to investigate the seroprevalence of antibodies to *T. gondii* among farmed wild boars in China. In an 11-month survey, a total of 882 serum samples were obtained from farmed wild boars from 3 cities (Jilin City, Siping City and Fusong City) in Jilin province, Northeast China and were tested for antibodies specific for *T. gondii*. Using modified agglutination test (MAT) and a cut-off titer of 1:25, the prevalence of *T. gondii* infection in the examined samples was 9.9% (88 of 882). The highest seroprevalence was observed in animals from Jilin city (15.3%, 43/281), followed by Siping (11.4%, 30/263) and Fusong (4.4%, 15/338). Logistic regression analysis revealed a significant correlation between the investigated geographic region and *T. gondii* infection. Also, prevalence was higher in females compared to males and the highest prevalnec was detected in piglets. These findings indicate that farmed wild boars may become a source of food-borne toxoplasmosis, posing a food safety threat to the public health in the investigated areas. Implementation of effective measures to control *T. gondii* infection in farmed wild boars in China may be warranted.

Key words: *Toxoplasma gondii* — Food safety — Seroprevalence — Risk factors — Wild boars — China

Introduction

Toxoplasma gondii, the causative agent of the disease toxoplasmosis, is a protozoan parasite that can infect virtually all warm-blooded animals and humans (Dubey, 2010; Calero-Bernal *et al*., 2016; de Souza *et al*., 2016; Gennari *et al*., 2016; Jiang *et al*., 2016). *T. gondii* has a complex lifecycle that encompasses sexual and asexual phases of reproduction. Sexual development occurs in the intestine of the felid, definitive hosts (Frenkel, 1973) and ends up with the formation and shedding of oocysts in the cat feces (Dubey, 2010). Asexual reproduction and formation of bradyzoites-containing tissue cysts occur in the tissues of the intermediate vertebrate host. Animals and humans acquire infection if they ingest food or water contaminated with oocysts (Dubey, 2004). Also, infection can be acquired congenitally through transplacental transmission of tachyzoites or postnatally via consumption of undercooked or raw meat, containing tissue cysts (Elsheikha, 2008; Dubey, 2009). In general, immunocompetent individual infected with *T. gondii* may develop flu-like symptoms. However, infection can cause abortion and stillbirth during pregnancy and fatal consequences in immunocompromised individuals (Montoya and Liesenfeld, 2004; Elsheikha, 2008; Wu *et al*., 2012a).

Toxoplasmosis is one of the most prevalent zoonotic parasitic diseases and is reported to be the third-leading cause of foodborne related deaths in the USA (Mead *et al*., 1999). Global seroprevalence of *T. gondii* varies from 1% to 100% depending on lifestyle, dietary habits (e.g. consumption of undercooked meat), environmental, socioeconomic, and hygienic conditions (Elsheikha, 2008; Furtado *et al*., 2011). In China, about 7.9% of the population is chronically infected with *T. gondii* and the number of infected people is projected to increase (Jiang *et al*., 2014). Unfortunately, there is no effective vaccine and anti-*T. gondii* chemotherapeutic drugs have limitations (Montoya and Liesenfeld, 2004; Serranti *et al*., 2011). The extremely braod host range, and the various routes and means by which *T. gondii*

can transmit among its hosts add more challenges to effective control of this ubiquitous parasite. Thus, while vaccination in humans or cats might be useful it would not completely solve the problem of *T. gondii* infection as long as parasite transmission between hosts is not prevented. Therefore, control strategies should also aim to minimize the parasite transmission from animals to humans, and this requires more understanding of *T. gondii* epidemiology by obtaining quantitative data on its prevalence.

Several studies on the prevalence of *T. gondii* in wild boars have been reported worldwide (Table 1) and *T. gondii* prevalence in pigs has been investigated in some regions of China (Table 2). Wild boar's meat is preferred in China because of its high nutritional value and palatable taste. In the meantime, wild pigs can serve as a vehicle for dissemination of *T. gondii* to humans (Choi *et al*., 1997). These facts suggest that wild boars can potentially serve as a source for human toxoplasmosis in China. There is a need to study the prevalence of *T. gondii* in farmed wild boars in China due to the potential public health impact. In an effort to improve the understanding of the epidemiology of toxoplasmosis in wild boars the present study was carried out to determine the seroprevalence and risk factors associated with *T. gondii* infection in farmed wild boars in three major cities (Jilin, Siping and Fusong) in Northeast China. Data about the occurrence of *T. gondii* in wild boars in China was discussed with respect to its implications for humans via the food chain and should help in any furure parasite risk management strategies.

Materials and Methods

Ethics statement

This study was approved by the Animal Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences. The wild boars from which

blood samples were collected were handled in strict accordance with good animal practices as stipulated in the Animal Ethics Procedures and Guidelines of the People's Republic of China.

Study population and sample collection

This research took place between April 2015 and February 2016, and aimed to assess the seroprevalence of *T. gondii* in free-ranged farmed wild boars foraging on mountain pasture in three Northeastern Chinese cities in Jilin province (Fig. 1), where about 500,000 farmed wild boars were raised. Farms based in Fusong city had a better farming and environmental conditions and the farm's location was far from residential areas. Sampling strategy was optimized in order to obtain a reliable estimate of the prevalence of *T. gondii* in the investigated regions.Therefore, based on prevalence (*P*) of *T. gondii* in farmed wild boars in Jilin Province of 19.2% (Xu *et al.*, 2015) with an accepted deviation of the true prevalence of 5% (*d*) and a confidence level of 95% (*z* = 1.96) the sample size was calculated as 247 [according to n = *P* (1 − *P*)*z*2/*d*2]. However, in the present study we aimed to examine more samples to maximize the reliability of the results. We randomly selected 882 wild boars from six piggeries in three cities: Jilin (*n* =281), Siping (*n* =263) and Fusong (*n* =338). Blood samples were collected from the precaval vein of wild boars using 18 gauge, 2.5" needles into 5 ml Vacutainer® blood collection tubes (Medical Equipment Factory, Liuyang City, Hubei Province). Sera were separated from blood samples in local veterinary stations and were transported to the laboratory and stored at −20°Cuntil analysis. Information regarding geographic origin, gender, age, and sampling time was collected for each sample.

Detection of anti-T. gondii antibodies

The modified agglutination test (MAT) was used to detect the antibodies against *T. gondii*, and the test was carried out as described previously (Dubey, 2010; Wang *et al*., 2016). MAT has an acceptable level of sensitivity and specificity for the detection of *T. gondii* antibodies and has been widely used in serological investigations in a wide range of animals worldwide (Gauss *et al*., 2005; Richomme *et al*., 2009; Richomme *et al*., 2010; Wu *et al*., 2012b; Coelho *et al*., 2014; Qin *et al*., 2015; Gennari *et al*., 2016; Wang *et al*., 2016;). The MAT was performed at serum dilutions of 1:10, 1:25, 1:100 and 1:500. Results are considered positive when obtained at dilution of ≥1:25. Antibody titers <1:25 were considered ‘‘suspect’’ and were retested (Gauss *et al*., 2005; Wang *et al*., 2016; Wu *et al*., 2016b). Positive and negative control sera were included in each test. In the present study, the cut-off of 1:25 was used in accordance with previous investigations (Dubey, 1997; Forbes *et al*., 20012) and because using more conservative cut-off may risk underestimating the actual prevalnce.

Statistical analysis

The variation in *T. gondii* seroprevalence (y) of wild boars by gender (x1), sampling time (x2), age (x3) and region (x4) were analyzed by $\chi 2$ test using SAS version 9.3 (SAS Institute Inc., USA). In the multivariable regression analysis, each of these variables was included in the binary Logit model as an independent variable. The best model was validated by Fisher’s scoring algorithm. All tests were two-sided, and when the probability (*P*) value < 0.05 the results were considered statistically significant. Odds ratios (ORs) and their 95% confidence intervals (95% CIs) were obtained to explore the strength of the association between *T. gondii*-seropositivity and the risk factors considered above.

Results and Discussion

The lack of both *T. gondii* surveillance analysis in wild boars and etiological diagnosis in meat-borne *T. gondii* infection has limited our knowledge about the transmission links of the wild boar-borne *T. gondii* infection in China. Therefore, we conducted a cross-sectional survey fromApril 2015 to February 2016 to evaluate the sero-prevalence of *T. gondii* infection in 882 farmed wild boars from three cities in Northeast China using the MAT assay.

Sero-prevalence data

The study showed that 88 (9.9%, 95% CI 8.00-11.96) of the 882 tested serum samples are seropositive to *T. gondii* tested with titers of 1:25 (n=69), 1:50 (n=15) and 1:100 (n=4) animals (Table 2), suggesting that wild boars are considered a potential source of exposure for humans in China. *T. gondii* seroprevalence reported in our study was lower than that reported in Finland 32.99% (65/197) (Jokelainen *et al*., 2012) and in Estonia 23.99% (113/471) (Jokelainen *et al*., 2015) by the direct agglutination test (DAT), and in Poland 37.60% (138/367) (Witkowski *et al*., 2015) by the multi-species ID Screen (MIDS). It is also lower than the 14-43.13% seroprevalence reported in wild boars in central Italy (Ranucci *et al*., 2013), Romania (Paştiu *et al*., 2013), Czech Republic (Bártová *et al*., 2006; Račka *et al*., 2015), Spain (Calero-Bernal *et al*., 2016), Sweden (Wallander *et al*., 2015), Korea (Kang *et al*., 2013), Latvia (Deksne *et al*., 2013), Spain (Gauss *et al*., 2005), Portugal (Coelho *et al*., 2014), Mediterranean island (Richomme *et al*., 2010), France (Richomme *et al*., 2009), and USA (Diderrich *et al*., 1996). On the other hand, sero-prevalence of *T. gondii* in our study was higher than that reported in Switzerland (6.67%, 10/150) (Berger-Schoch *et al*., 2011), but

similar to that reported in Slovak Republic (8.13%, 26/320) (Antolová *et al*., 2007) tested by ELISA, and in Japan (6.29%, 11/175) (Matsumoto *et al*., 2011) by the latex agglutination test (LAT) (Table 1). Interestingly, the wild boars investigated in our study had lower *T. gondii* seroprevalence compared with the remarkably high 11.26-70.00% *T. gondii* seroprevalence reported in intensive pig farms in China (Table 1). Interestingly, the relatively higher prevalence reported in some studies in Europe comapred to China (Table 1) could be due to the fact that wild boars investigated in Europe were free ranging and thus have more opportunities to encounter the infection than the investigated animals in our study.

Risk factors

The effect of multiple variables on *T. gondii*-seropositivity was assessed by stepwise logistic regression analysis using Fisher's test. In the final model, only the geographic locality from which sera were collected had a significant effect on the risk of *T. gondii* infection (OR=1.876, 95% CI 1.418-2.482). Wild boars tested from Fusong City (4.4%, 15/338; 95% CI 2.24-6.63) had the lowest *T. gondii* seroprevalence compared to animals tested from Jilin City (15.3%, 43/281; 95% CI 11.09-19.51) and Siping City (11.4%, 30/263; 95% CI 7.57-15.25); this difference was statistically significant (P<0.0001) (Table 2). *T. gondii* transmission cycle in a piggery may be perpetuated by multiple factors, including (i) the contamination of the environment with oocyst from cat feces and (ii) increased susceptibility to infection in piglets due to immature immune defenses. Hence, large numbers of stray cats live in Jilin and Siping may be one of the reasons why seroprevalence of wild boars tested from Jilin and Siping was higher than that of animals from Fusong. This is not surprising, as cats are definitive hosts of *T. gondii* and oocysts secreted by cats represent a major source of *T. gondii* infection to wild boars. It is known that the prevalence of *T. gondii* in pigs can be

affected by management systems where in poorly managed systems, seroprevalence in pigs can reach 68% (Gamble *et al*., 1999). Therefore, the better farming and environmental conditions (i.e. less contamination with oocysts) of wild boars in Fusong may have contributed to the reduction in the frequency by which intermediate host (boars) have access to oocysts of the definitive host, ultimately leading to a lower seroprevalence in wild boars from Fusong.

Also, in this tsudy we determined the prevelance of *T. gondii* in males and females, which was found to be 5.34% and 10.79%, respectively (Table 2). This finding is similar to that of previous studies in mice where female mice appeared to be more susceptible to infection with *T. gondii* than male mice (Roberts *et al*., 1995). However, other studies have not reported any correlation betwen the gender of pigs and anti-*T. gondii* seropositivity (Alvarado-Esquivel *et al*., 2015; Gebremedhin *et al*., 2015). In addition to gender-related differences, we studied *T. gondii* seroprevalence in different age groups, which was found to range from 5.38% to 30.30%. The highest seroprevalence tend to be in young piglets (<22 days) and with increasing age this gradually declined by as much as 6-fold for the prevalence in pigs >66 days old. (Table 2). This pattern of early acquisition followed by a gradual decline in prevalence is probably due to different husbandry practice, outdoor piggery density or increased susceptibility in young pigs ((Dubey, 1986).

Some limitations should be highlighted. First, the study design did not include collection of meat samples and hence we were not able to isolate any *T. gondii* strains for subsequent genotyping characterization. Second, we were unable to conclusively confirm the associations between geographic region, gender, age, year of study and anti-*T. gondii* seropositivit in the multivariate analysis, probably due to the small (n = 882) and unbalanced sample size (619 and

263 in the year 2015 and 2016, respectively). Testing 882 animals from three cities is not representative of the prevalence of *T. gondii* in farmed wild boar population in China. The exact basis for association between geographic region, age, gender, year of study remains to be elucidated. It is noteworthy that none of the previous prevalence studies or the present study have conducted direct statistical comparisons between pig populations from different regions and countries. This is probably due to the difficulty associated with controlling the confounding effects caused by differences in pig populations, designs and methodologies of the different studies, such as sampling site, sampling strategy, the type and age of pig included in the survey, specificity and sensitivity of the detection methods, and ecological and geographical factors.

In conclusion, we have estimated the prevalence of *T. gondii* in wild farmed boars in Northeast China, which provides new information for assessment of human exposure to *T. gondii* through consumption of wild boar's meat. To our knolwdge, our study is the first to demonstrate the presence of *T. gondii* infection in (9.9%) farmed wild boars in China, with the highest seroprevalence in Jilin (15.3%), followed by Siping (11.4%) and Fusong (4.4%). Logistic regression analysis indicated that region is a risk factor associated with *T. gondii* infection in the investigated wild boar populations. These results highlight the importance of *T. gondii* in farmed wild boars as a potential pathogen to be considered in the control of food-borne infection risks. The isolation of multiple strains of *T. gondii* known to cause disease in humans would suggest that wild boars are a source/reservoir for *T. gondii* infection in humans in China, although this requires further study. In the future, more research is needed to isolate and characterize the genotype of *T. gondii* strains present in farmed wild boars in order to dissociate the role of wild boars in food-borne *T. gondii* infection and to

differentiate foodborne illnesses related to consumption of wild boars from those related to consumption of different types of meat.

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No competing financial interests exist. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the funding agencies.

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TABLE 1. GLOBAL PREVALENCE OF *TOXOPLASMA GONDII* INFECTION IN WILD BOARS.

	Region	*Test*[a]	*No. positive/ no. tested*	*Prevalence (%)*	*Year*	*Reference*
Europe	Spain	ELISA	688/2881	23.8	2003-2011	Calero-Bernal *et al*., 2016
	Spain	MAT	185/517	38.4	1993-2004	Gauss *et al*., 2005
	Portugal	MAT	20/97	20.6	2011-2012	Coelho *et al*., 2014
	Estonia	DAT	113/471	23.9	2012-2013	Jokelainen *et al*., 2015
	Poland	MIDS	138/367	37.6	2009-2011	Witkowski *et al*., 2015
	Sweden	ELISA	207/480	43.1	2005-2011	Wallander *et al*., 2015
	Central Italy	IFAT	56/400	14	2009-2011	Ranucci *et al*., 2013
	USA	MAT	33/108	30.5	1990	Diderrich *et al*., 1996
	Romania	IFAT	24/150	16	2008-2010	Paştiu *et al*., 2013
	Latvia	ELISA	201/606	33.1	2010-2011	Deksne *et al*., 2013
	Finland	DAT	65/197	32.9	2007-2008	Jokelainen *et al*., 2012
	Switzerland	ELISA	10/150	6.6	2006-2008	Berger-Schoch *et al*., 2011
	Slovak Republic	ELISA	26/320	8.1	2003	Antolová *et al*., 2007
	Mediterranean island	MAT	566/1399	40.4	2006-2007	Richomme *et al*., 2010
	France	MAT	26/148	17.5	2002–2008	Richomme *et al*., 2009
	The Czech Republic	ELISA	148/565	26.1	1999-2005	Bártová *et al*., 2006
	The Czech Republic	ELISA	260/656	39.6	2008-2010	Račka *et al*., 2015
	Greek	ELISA	5/94	5.2	2006-2010	Touloudi *et al*.,2015
Asia	Korea	ELISA	131/521	25.1	2009-2011	Kang *et al*., 2013
	Japan	LAT	11/175	6.2	2004-2007	Matsumoto *et al*., 2011
	Guizhou, China	ELISA	49/70	70	2011-2012	Li et al., 2015

Liaoning, China	MAT	233/2063	11.2	2013-2014	Wang et al., 2016
Jilin, China	IHA	236/1235	19.1	2013	Xu et al., 2015
Hunan, China	IHA	373/1191	31.3	2010-2012	Xu et al., 2014
Jiangxi, China	IHA	282/1232	22.8	2012	Jiang et al., 2014
Heilongjiang, China	IHA	47/1,014	4.6	2011-2012	Chang et al., 2013
Chongqing, China	IHA	278/908	30.6	UN[b]	Wu et al., 2012a
Liaoning, China	IHA	140/1,164	12.03	2011	Liu et al., 2012
Tibet, China	MAT	97/427	22.7	2010	Wu et al., 2012b
Zhejiang, China	ELISA	434/813	53.3	2009-2010	Yu et al., 2011
Central China	IHA	873/3,558	24.5	2008-2009	Tao et al., 2011
Guangdong, China	ELISA	276/1,022	27.01	2008-2009	Zhou et al., 2010
Fujian, China	IHA	87/605	14.3	2006-2007	Huang et al., 2010
Yunnan, China	IHA	141/831	16.9	2008-2009	Zou et al., 2009

[a] ELISA, enzyme-linked immunosorbent assay; IHA, indirect hemagglutination test; MAT, modified agglutination test; DAT, direct agglutination test; LAT, latex agglutination test; IFAT, immunofluorescence antibody test; MIDS, multi-species ID Screen.

[b] UN, unknown.

TABLE 2. SEROPREVALENCE OF *TOXOPLASMA GONDII* INFECTION IN WILD BOARS IN THREE CITIES IN NORTHEAST CHINA BY REGION, GENDER, AGE AND COLLECTION YEAR.

Variable	*Category*	*Sera with different MAT titers*			*No. tested*	*No. positive*	*Prevalence (%) (95%CI)*	*P-value*	*OR (95% CI)*
		1:25	*1:50*	*1:100*					
Region	Fusong	11	2	2	338	15	4.4 (2.24-6.63)	<0.0001	1.0 (Reference)
	Siping	25	5	0	263	30	11.4 (7.57-15.25)		2.77 (1.46-5.27)
	Jilin	33	8	2	281	43	15.3 (11.09-19.51)		3.89 (2.11-7.17)
Gender	Male	6	1	0	131	7	5.3 (1.49-9.20)	0.0551	1.0 (Reference)
	Female	63	14	4	751	81	10.7 (8.57-13.00)		2.14 (0.97-4.75)
Age	<22 days[a]	8	2	0	33	10	30.3 (14.62-45.98)	<0.0001	1.0 (Reference)
	22-66 days	6	4	2	233	12	5.3(2.42_8.34)		7.65 (2.98-19.63)
	>66 days	55	9	2	626	66	10.5 (8.14-12.95)		2.07 (1.10-3.91)
Collection year	2015	441	10	4	619	58	9.3 (7.07-11.67)	0.3558	1.0 (Reference)
	2016	25	5	0	263	30	11.4 (7.57-15.25)		1.25 (0.78-1.99)
Total		69	15	4	882	88	9.98 (8.00-11.96)		

[a] pre-weaned.

Figure Legend:

Fig. 1. Map showing three cities, Jilin, Siping and Fusong, in Jilin province, Northeast China where the serum samples have been collected.

Address correspondence to:
Xiao-Xuan Zhang PhD and *Xing-Quan Zhu PhD*
State Key Laboratory of Veterinary Etiological Biology,
Key Laboratory of Veterinary Parasitology of Gansu Province,
Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences,
Lanzhou, Gansu Province 730046, PR China
E-mail: zhangxiaoxuan1988@126.com (X.X. Zhang);
xingquanzhu1@hotmail.com (X.Q. Zhu)

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