

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

2 Northeast China

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21 **Abstract**

22 The apicomplexan protozoan parasite *Toxoplasma gondii* is a widely distributed etiological
23 agent of food-borne illness. This parasite can cause production losses in livestock and serious
24 disease in humans through consumption of contaminated meat. Pig's meat is the most likely
25 source of human infection and wild boars may play a role in the transmission of *T. gondii* by
26 serving as a reservoir host. This study aimed to investigate the seroprevalence of antibodies to
27 *T. gondii* among farmed wild boars in China. In an 11-month survey, a total of 882 serum
28 samples were obtained from farmed wild boars from 3 cities (Jilin City, Siping City and
29 Fusong City) in Jilin province, Northeast China and were tested for antibodies specific for *T.*
30 *gondii*. Using modified agglutination test (MAT) and a cut-off titer of 1:25, the prevalence of
31 *T. gondii* infection in the examined samples was 9.9% (88 of 882). The highest
32 seroprevalence was observed in animals from Jilin city (15.3%, 43/281), followed by Siping
33 (11.4%, 30/263) and Fusong (4.4%, 15/338). Logistic regression analysis revealed a
34 significant correlation between the investigated geographic region and *T. gondii* infection.
35 Also, prevalence was higher in females compared to males and the highest prevalence was
36 detected in piglets. These findings indicate that farmed wild boars may become a source of
37 food-borne toxoplasmosis, posing a food safety threat to the public health in the investigated
38 areas. Implementation of effective measures to control *T. gondii* infection in farmed wild
39 boars in China may be warranted.

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

44

45 **Introduction**

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

61 Toxoplasmosis is one of the most prevalent zoonotic parasitic diseases and is reported to
62 be the third-leading cause of foodborne related deaths in the USA (Mead *et al.*, 1999). Global
63 seroprevalence of *T. gondii* varies from 1% to 100% depending on lifestyle, dietary habits
64 (e.g. consumption of undercooked meat), environmental, socioeconomic, and hygienic
65 conditions (Elsheikha, 2008; Furtado *et al.*, 2011). In China, about 7.9% of the population is
66 chronically infected with *T. gondii* and the number of infected people is projected to increase
67 (Jiang *et al.*, 2014). Unfortunately, there is no effective vaccine and anti-*T. gondii*
68 chemotherapeutic drugs have limitations (Montoya and Liesenfeld, 2004; Serranti *et al.*,
69 2011). The extremely broad host range, and the various routes and means by which *T. gondii*

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

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

89

90 **Materials and Methods**

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92 *Ethics statement*

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

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

97

98 *Study population and sample collection*

99 This research took place between April 2015 and February 2016, and aimed to assess the
100 seroprevalence of *T. gondii* in free-ranged farmed wild boars foraging on mountain pasture
101 in three Northeastern Chinese cities in Jilin province (Fig. 1), where about 500,000 farmed
102 wild boars were raised. Farms based in Fusong city had a better farming and environmental
103 conditions and the farm's location was far from residential areas. Sampling strategy was
104 optimized in order to obtain a reliable estimate of the prevalence of *T. gondii* in the
105 investigated regions. Therefore, based on prevalence (P) of *T. gondii* in farmed wild boars in
106 Jilin Province of 19.2% (Xu *et al.*, 2015) with an accepted deviation of the true prevalence of
107 5% (d) and a confidence level of 95% ($z = 1.96$) the sample size was calculated as 247
108 [according to $n = P(1 - P)z^2/d^2$]. However, in the present study we aimed to examine more
109 samples to maximize the reliability of the results. We randomly selected 882 wild boars from
110 six piggeries in three cities: Jilin ($n = 281$), Siping ($n = 263$) and Fusong ($n = 338$). Blood
111 samples were collected from the precaval vein of wild boars using 18 gauge, 2.5" needles into
112 5 ml Vacutainer® blood collection tubes (Medical Equipment Factory, Liuyang City, Hubei
113 Province). Sera were separated from blood samples in local veterinary stations and were
114 transported to the laboratory and stored at -20°C until analysis. Information regarding
115 geographic origin, gender, age, and sampling time was collected for each sample.

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117

118 *Detection of anti-T. gondii antibodies*

119 The modified agglutination test (MAT) was used to detect the antibodies against *T.*
120 *gondii*, and the test was carried out as described previously (Dubey, 2010; Wang *et al.*, 2016).
121 MAT has an acceptable level of sensitivity and specificity for the detection of *T. gondii*
122 antibodies and has been widely used in serological investigations in a wide range of animals
123 worldwide (Gauss *et al.*, 2005; Richomme *et al.*, 2009; Richomme *et al.*, 2010; Wu *et al.*,
124 2012b; Coelho *et al.*, 2014; Qin *et al.*, 2015; Gennari *et al.*, 2016; Wang *et al.*, 2016;). The
125 MAT was performed at serum dilutions of 1:10, 1:25, 1:100 and 1:500. Results are considered
126 positive when obtained at dilution of $\geq 1:25$. Antibody titers $< 1:25$ were considered “suspect”
127 and were retested (Gauss *et al.*, 2005; Wang *et al.*, 2016; Wu *et al.*, 2016b). Positive and
128 negative control sera were included in each test. In the present study, the cut-off of 1:25 was
129 used in accordance with previous investigations (Dubey, 1997; Forbes *et al.*, 20012) and
130 because using more conservative cut-off may risk underestimating the actual prevalence.

131

132 *Statistical analysis*

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

141

142 **Results and Discussion**

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

149

150 *Sero-prevalence data*

151 The study showed that 88 (9.9%, 95% CI 8.00-11.96) of the 882 tested serum samples are
152 seropositive to *T. gondii* tested with titers of 1:25 (n=69), 1:50 (n=15) and 1:100 (n=4)
153 animals (Table 2), suggesting that wild boars are considered a potential source of exposure for
154 humans in China. *T. gondii* seroprevalence reported in our study was lower than that reported
155 in Finland 32.99% (65/197) (Jokelainen *et al.*, 2012) and in Estonia 23.99% (113/471)
156 (Jokelainen *et al.*, 2015) by the direct agglutination test (DAT), and in Poland 37.60%
157 (138/367) (Witkowski *et al.*, 2015) by the multi-species ID Screen (MIDS). It is also lower
158 than the 14-43.13% seroprevalence reported in wild boars in central Italy (Ranucci *et al.*,
159 2013), Romania (Paștiu *et al.*, 2013), Czech Republic (Bártová *et al.*, 2006; Račka *et al.*,
160 2015), Spain (Calero-Bernal *et al.*, 2016), Sweden (Wallander *et al.*, 2015), Korea (Kang *et*
161 *al.*, 2013), Latvia (Deksne *et al.*, 2013), Spain (Gauss *et al.*, 2005), Portugal (Coelho *et al.*,
162 2014), Mediterranean island (Richomme *et al.*, 2010), France (Richomme *et al.*, 2009), and
163 USA (Diderrich *et al.*, 1996). On the other hand, sero-prevalence of *T. gondii* in our study was
164 higher than that reported in Switzerland (6.67%, 10/150) (Berger-Schoch *et al.*, 2011), but

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

173

174 *Risk factors*

175 The effect of multiple variables on *T. gondii*-seropositivity was assessed by stepwise
176 logistic regression analysis using Fisher's test. In the final model, only the geographic locality
177 from which sera were collected had a significant effect on the risk of *T. gondii* infection
178 (OR=1.876, 95% CI 1.418-2.482). Wild boars tested from Fusong City (4.4%, 15/338; 95%
179 CI 2.24-6.63) had the lowest *T. gondii* seroprevalence compared to animals tested from Jilin
180 City (15.3%, 43/281; 95% CI 11.09-19.51) and Siping City (11.4%, 30/263; 95% CI
181 7.57-15.25); this difference was statistically significant ($P<0.0001$) (Table 2). *T. gondii*
182 transmission cycle in a piggery may be perpetuated by multiple factors, including (i) the
183 contamination of the environment with oocyst from cat feces and (ii) increased susceptibility
184 to infection in piglets due to immature immune defenses. Hence, large numbers of stray cats
185 live in Jilin and Siping may be one of the reasons why seroprevalence of wild boars tested
186 from Jilin and Siping was higher than that of animals from Fusong. This is not surprising, as
187 cats are definitive hosts of *T. gondii* and oocysts secreted by cats represent a major source of
188 *T. gondii* infection to wild boars. It is known that the prevalence of *T. gondii* in pigs can be

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

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

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

212 263 in the year 2015 and 2016, respectively). Testing 882 animals from three cities is not
213 representative of the prevalence of *T. gondii* in farmed wild boar population in China. The exact
214 basis for association between geographic region, age, gender, year of study remains to be
215 elucidated. It is noteworthy that none of the previous prevalence studies or the present study
216 have conducted direct statistical comparisons between pig populations from different regions
217 and countries. This is probably due to the difficulty associated with controlling the confounding
218 effects caused by differences in pig populations, designs and methodologies of the different
219 studies, such as sampling site, sampling strategy, the type and age of pig included in the survey,
220 specificity and sensitivity of the detection methods, and ecological and geographical factors.

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

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

236

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245

246 **Author Disclosure Statement**

247 No competing financial interests exist. The findings and conclusions in this report are
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249

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

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

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423 ^aELISA, enzyme-linked immunosorbent assay; IHA, indirect hemagglutination test; MAT, modified agglutination test; DAT, direct agglutination
424 test; LAT, latex agglutination test; IFAT, immunofluorescence antibody test; MIDS, multi-species ID Screen.

425 ^bUN, unknown.

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

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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)		

429 ^a pre-weaned.

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437 **Figure Legend:**

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

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