A Seroepidemiological Survey of *Toxoplasma gondii* Infection in Free-Range and Caged Ducks in Southwest China

Zhao, G.,[1] Song, Z.,[1] Wang, S.,[2] Hassan, I.A.,[2] Wang, W.,[2] Cheng, F.[1] and Yang, X.[1]*

[1] Department of Veterinary Medicine, Southwest University, Chongqing, 402460, PR China.
[2] College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, Jiangsu, 210095, PR China.

* **Corresponding Author:** Dr. X. Yang, Department of Veterinary Medicine, Southwest University, Chongqing, 402460, PR China. Tel.: +86 23 46751041, Fax: +86 23 46751041. E-mail: yangxiaowei396@163.com

ABSTRACT

Toxoplasma gondii is widely distributed in humans and other animals including domestic poultry throughout the world, but data on prevalence of *T. gondii* in ducks in People's Republic of China (PRC), especially in the southwest China are limited. In the present study, the seroprevalence of *T. gondii* infection in free-range (FR) and caged ducks was investigated in Chongqing municipality (southwest China) during the period from February to July 2013. A total of 1162 serum samples including 635 FR ducks and 527 caged ducks from 8 municipal districts/counties including Yongchuan, Dazu, Rongchang, Hechuan, Liangping, Wanzhou, Yunyang and Kaixian were collected and assayed for *T. gondii* antibodies using modified agglutination test (MAT) technique. Partial seropositive samples were validated for *T. gondii* by bioassay in mice. The prevalence in FR and caged ducks was 13.23% and 6.64% respectively. Statistical analysis showed that FR group was significantly higher than caged group ($p<0.05$). From 12 FR ducks in Rongchang, one strain of *T. gondii* was successfully isolated and the isolation rate was 8.33% (1/12). This is the first report of isolation of *T. gondii* from FR ducks in China.

Keywords: Duck; *Toxoplasma gondii*; Prevalence; China

INTRODUCTION

Toxoplasma gondii is an important intracellular protozoan parasite, widely prevalent in humans and animals, including ducks throughout the world (1-4). Felids are the definitive host of this parasite and almost all warm-blooded animals including humans can act as intermediate hosts (5). Humans can be infected by ingesting tissue cysts from under-processed infected animal meat or from food or drink contaminated with oocysts shed in cat feces (6). Some early studies demonstrated that meat from *T. gondii*-infected poultry (including chickens, ducks, geese, and pigeons) was the primary risk factor for humans' toxoplasmosis (1, 7, 8). Ducks (free-range (FR) and caged), are consumed widely in many countries, including China, especially in Chongqing, southwest China. In addition individuals have the habit of eating under-cooked duck intestines called "chafing dish". This further emphasizes the need to study the prevalence of *T. gondii* in ducks.

In recent years sero-prevalence studies of *T. gondii* in ducks have been conducted extensively in various parts of the world (9-11). Surveys have also been conducted in the mainland China including northeast China (8), southeast China (12) and northwest China (13). However, little is known about the prevalence of *T. gondii* in ducks in southwest China. Here, we report for the first time *T. gondii* sero-prevalence in FR and caged ducks in Chongqing municipality, southwest China.

MATERIALS AND METHODS

The study area

The study was conducted in Chongqing municipality which is located in the upper reaches of the Yangtze River in the

southwest part of mainland China, covering an area of 82,000 km^2 and a population of approximately 2.94 million. Its geographical position is at east longitude 105˚11' - 110˚11' and at north latitude 28˚10' - 32˚13'. The area has a subtropical humid climate, a long winter and summer, with a brief spring and autumn. The average annual temperature is 18.0˚C (in winter the average is 6-8˚C, in summer the average is above 35˚C), with a mean annual rainfall of 1000-1450mm. There are 19 municipal districts and 19 municipal counties distributed in the Chongqing municipality.

Eight municipal districts or counties including Yongchuan, Dazu, Rongchang, Hechuan, Liangping, Wanzhou, Yunyang and Kaixian, located in the western and eastern parts of Chongqing municipality, were selected for sample collections. All of the above locations are main suppliers of duck meat to Chongqing and the neighboring regions.

Blood samples

A total of 1162 blood samples from adult ducks, 635 blood samples from free-range (FR) ducks and 527 blood samples from caged ducks were collected from the above eight places in Chongqing between February and July 2013. The blood samples were sent to the laboratory for serological examination and centrifuged at 3,000 rpm for 10 minutes and the sera were stored at –20˚C until tested for antibodies to *T. gondii*.

Serological assay

Sera were tested for *T. gondii* antibodies using 2-fold serial dilutions from 1:25 to 1:3,200 with the modified agglutination test (MAT), as described previously (8, 14). Briefly, the harvested parasites were kept in 6% formaldehyde solution at 4˚C overnight, and suspended in the alkaline buffer at 20,000 parasites/mL. Two-fold dilutions of sera were performed using the serum diluting buffer, and agglutination was performed in U-bottom 96-well microtiter plates using a mixture of 50 µL antigen and 50 µL diluted sera. The plates were incubated at 37˚C overnight. The test was considered positive when a layer of agglutinated parasites was formed in wells at dilutions of 1:25 or higher; positive and negative controls were included in each test.

Bioassay of ducks for *T. gondii*

Tissues for bioassay were sampled from the MAT sero-positive ducks as described previously (15). Brains, hearts, spleens, lungs, livers and kidneys of ducks were bioassayed individually in outbred female Swiss Webster (SW) mice (20±2g) obtained from Center of Laboratory Animals, Chongqing Medical University. (Chongqing, PR China). In this research, all the animals used were submitted to protocols approved by the Animal Care and Ethics Committee of Southwest University (Approval No. 201209025).

Identification of the isolate by PCR

Molecular identification of the isolate was performed with a 341bp fragment of the internal transcribed spacer 1(ITS-1) gene by a pair of primers 5'-AGTTTAGGAAGCAATCTGAAAGCACATC-3' and 5'-GATTTGCATTCAAG AAGCGTGATAGTAT-3' as described previously (16). Briefly, total genomic DNA was extracted from the peritoneal washings with a commercially available DNA extraction kit (Geneaid Biotech Ltd., New Taipei City, Taiwan) according to the manufacturer's recommendations. PCR reactions (25µl) were performed in 2.5mM of $MgCl_2$, 0.4µM of each primer, 2.5µl 1×rTaq buffer, 0.25mM of each desoxyribonucleotide, 0.625 U of rTaq DNA polymerase (TaKaRa), and 3µl of DNA sample in a thermocycler (Biometra) under the following conditions: after an initial denaturation at 94˚C for 5 minutes, then 35 cycles of 94˚C for 30 s (denaturation), 55˚C for 30 s (annealing), 72˚C for 30 s (extension), followed by a final extension at 72˚C for 7 minutes. The positive control of *T. gondii* DNA and negative control (no-DNA control) were included in each amplification run. Each amplicon (13ul) was examined by agarose gel electrophoresis to validate amplification efficiency.

Statistical analysis

Statistical analyses of *T. gondii* prevalence between free-range and caged groups were performed by χ^2-test using Excel (Microsoft® Excel 2003). The differences were considered statistically significant where $p < 0.05$.

RESULTS

Positive ratios for FR and caged ducks and the overall ratios for the eight municipal districts/counties in Chongqing

A total of 1162 duck serum samples including 635 FR and 527 caged ducks from 8 municipal districts/counties of Chongqing were tested for *T. gondii* antibodies by MAT

method. Overall, the seroprevalence of *T. gondii* in FR ducks was 13.23%, which was significantly higher ($p<0.05$) than that of the caged ducks (6.64%) (Table 1).

Seropositivity rates for the FR ducks from the 8 municipal districts/counties ranged from 7.14% to 19.51% (Table 1). Four samples out of 56 tested was found positive (7.14%) in Wanzhou which was statistically lower compared to Dazu ($p<0.05$) and Hechuan ($p<0.05$). The positive rates ranged from 10.14% to 19.51% in Dazu, Hechuan, Yongchuan, Rongchang, Liangping, Yunyang and Kaixian municipal districts/counties. However, there were no significant differences between them ($p>0.05$).

For caged ducks, the positive rates of the 8 municipal districts/counties ranged from 4.69% to 9.43% (Table 1). No significant differences were found between the eight municipal districts/counties ($p>0.05$).

Isolation of *T. gondii* from the seropositive ducks

Twelve FR ducks from Rongchang with titers of 1:25 in 6 ducks, 1:50 in 3 ducks, and 1:100 in 3 ducks were used for *T. gondii* isolation. At the third blind passages of the surviving mice, one strain of *T. gondii* was isolated from a duck with titer 1:100. Tachyzoites were found in peritoneal washings of all of the 4 mice inoculated (Figure 1). However, the rest of the mice inoculated with samples from other ducks were negative for *T. gondii*. Tissue cysts were not found in the brain squashes of any of the mice. The isolation rate was 8.33% (1/12).

Identification of the isolate by PCR

The isolate was identified by PCR using a 341bp fragment of the ITS-1 gene as a target (16). Alignment of nucleic acid sequences of PCR product shared 100% similarity to RH strain of *T. gondii* (accession No. U16161) homologues, confirmed that it belonged to *T. gondii*.

Table 1: Seroprevalence of *Toxoplasma gondii* infection in ducks in Chongqing, southwestern China by the modified agglutination test (MAT)

Places		No tested	No. with anti-*T. gondii* antibodies					Total positive	Prevalence (%)
			1: 25	1: 50	1: 100	1: 200	1: 400		
Yongchuan	FR	77	7	1	1	0	0	9	11.69
	Caged	65	4	1	0	0	0	5	7.69
	Total	142	11	2	1	0	0	14	9.86
Dazu	FR	82	11	2	1	1	1	16	19.51
	Caged	70	5	1	0	0	0	6	8.57
	Total	152	16	3	1	1	1	22	14.47
Rongchang	FR	88	6	3	3	0	0	12	13.64
	Caged	72	2	1	1	0	0	4	5.56
	Total	160	8	4	4	0	0	16	10.00
Hechuan	FR	66	4	4	2	1	1	12	18.18
	Caged	53	2	1	1	1	0	5	9.43
	Total	119	6	5	3	2	1	17	14.29
Liangping	FR	90	8	2	2	0	0	12	13.33
	Caged	89	4	1	0	0	0	5	5.62
	Total	179	12	3	2	0	0	17	9.50
Wanzhou	FR	56	3	1	0	0	0	4	7.14
	Caged	55	3	0	0	0	0	3	5.45
	Total	111	6	1	0	0	0	7	6.31
Yunyang	FR	69	4	1	1	1	0	7	10.14
	Caged	64	2	1	0	0	0	3	4.69
	Total	133	6	2	1	1	0	10	7.52
Kaixian	FR	107	5	4	2	1	0	12	11.21
	Caged	59	3	2	0	0	0	5	8.47
	Total	166	8	6	2	1	0	17	10.24
Total FR		635	48	18	12	4	2	84	13.23
Total caged		527	25	8	2	0	0	35	6.64
Total		1162	73	26	14	4	2	109	9.38

FR: Free-range

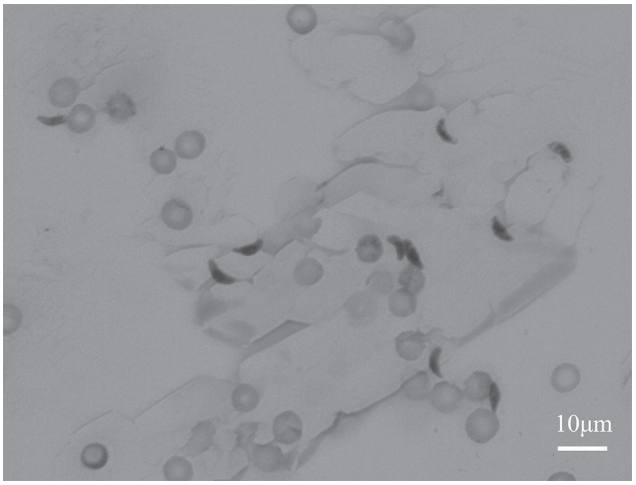

Figure 1: Tachyzoites in the peritoneal washings of SW mice. (Giemsa stained, Magnification × 1000).

DISCUSSION

Many surveys detecting *T. gondii* infection in FR ducks have been conducted in a number of other countries (4, 8, 12, 17, 18), and the prevalence rates in most of these studies ranged between 21.2% to 50.0%. In China, the *T. gondii* seroprevalence in FR ducks were also performed in north-eastern China by Yang *et al.* (8), in south-eastern China by Yan *et al.* (12) and in northwest China by Cong *et al.* (13). The seropositive rates in that three reports were 12.3%, 16% and 13.90%, respectively. In the present study, the overall *T. gondii* seroprevalence in FR ducks in Chongqing was 13.2%, which was lower than that reported in other countries but similar to that reported in China. The differences in seroprevalence between China and other countries may due to differences in ecological and geographical factors.

Considering the caged ducks, the seroprevalence in the present study was 6.64% which was also similar to that reported in north-eastern China (7.50%) (8) and north-western China (6.31%) (13). These findings indicated that the prevalence rates of *T. gondii* in caged ducks were approximately equal in various regions of China. When comparing the caged and FR ducks in this study, the seroprevalence of *T. gondii* in caged group (6.64%) was significantly lower than the FR group (13.23%) ($p<0.05$). The main reason for this variability could be that the FR ducks were fed on the ground and had more chance than the caged ducks to be infected by ingesting *T. gondii* oocysts from the environment, such as water, soil and infected tissues from intermediate hosts. Our findings were in agreement with the results obtained by Dubey *et al.* (10, 19, 20) in *T. gondii* infected FR chickens.

Geographically, there are 38 municipal districts/counties distributed in the Chongqing municipality and eight places were selected for screening the *T. gondii* seroprevalence because they were the main suppliers of duck meat. The positive rate of FR ducks in Wanzhou (7.14%) was found statistically lower than Dazu (19.51%) and Hechuan (18.18%). Reasons for this difference could be varied, including the sampling season, the quantity of *T. gondii* oocysts and the number of samples. Duck meat can serve as a source of *T. gondii* infection for human and other animals; therefore it would be a risk factor for toxoplasmosis of human and other animals; awareness of this fact should be emphasized.

To validate the seropositive samples, 12 FR ducks from Rongchang were used to bioassay the *T. gondii* from the tissues and one strain was successfully isolated. To the best of our knowledge, this is the first report of the successful isolation from FR ducks in China. Successful isolation of *T. gondii* from tissues of asymptomatic animals may depend on the number of mice inoculated, the amount of tissue-bioassayed and the concentration of the parasite in sampled tissues (21, 22). In this study, the isolation rate (8.33%) was lower than previously reported (19, 23). The reason may be due to less tissue cysts in the processed samples. Furthermore, the genetic characterization of the isolate was not performed in this study and requires further investigation.

ACKNOWLEDGEMENTS

This work was supported by Fundamental Research Funds for the Central Universities (XDJK2012C100) and The Doctoral Special Funding of SWU (2013Bsr09).

REFERENCES

1. Dubey, J.P.: *Toxoplasma gondii* infections in chickens (Gallus domesticus): prevalence, clinical disease, diagnosis and public health significance. Zoonoses Public Health. 57: 60-73, 2010.
2. Zhou, P., Chen, Z., Li, H.L., Zheng, H., He, S., Lin, R.Q. and Zhu, X.Q.: *Toxoplasma gondii* infection in humans in China. Parasit vectors. 4: 165, 2011.
3. Zhao, G., Shen, B., Xie, Q., Xu, L.X., Yan, R.F., Song, X.K., Hassan, I.A. and Li, X.R.: Detection of *Toxoplasma gondii* in free-range chickens in China based on circulating antigens and antibodies. Vet.Parasitol. 185: 72-77, 2012.
4. El-Massry, A., Mahdy, O.A., El-Ghaysh, A. and Dubey, J.P.: Prevalence of *Toxoplasma gondii* antibodies in sera of turkeys, chickens, and ducks from Egypt. J. Parasitol. 86: 627-628, 2000.

5. Dubey, J.P.: Toxoplasmosis. J. Am. Vet. Med. Assoc. 189: 166-170, 1986.
6. Schlundt, J., Toyofuku, H., Jansen, J. and Herbst, S.A.: Emerging food-borne zoonoses. Rev. Sci. Tech. 23: 513-533, 2004.
7. Dubey, J.P., Webb, D.M., Sundar, N., Velmurugan, G.V., Bandini, L.A., Kwok, O.C. and Su, C.: Endemic avian toxoplasmosis on a farm in Illinois: clinical disease, diagnosis, biologic and genetic characteristics of *Toxoplasma gondii* isolates from chickens (*Gallus domesticus*), and a goose (*Anser anser*). Vet. Parasitol. 148: 207-212, 2007.
8. Yang, N., Mu, M.Y., Li, H.K., Long, M. and He, J.B.: Seroprevalence of T*oxoplasma gondii* infection in slaughtered chickens, ducks, and geese in Shenyang, northeastern China Parasit. Vectors. 5: 237, 2012.
9. Maksimov, P., Buschtons, S., Herrmann, D.C., Conraths, F.J., Gorlich, K., Tenter, A.M., Dubey, J.P., Nagel-Kohl, U., Thoms, B., Botcher, L., Kuhne, M. and Schares, G.: Serological survey and risk factors for *Toxoplasma gondii* in domestic ducks and geese in Lower Saxony, Germany. Vet. Parasitol. 182: 140-149, 2011.
10. Dubey, J.P., Graham, D.H., Dahl, E., Hilali, M., El-Ghaysh, A., Sreekumar, C., Kwok, O.C., Shen, S.K. and Lehmann, T.: Isolation and molecular characterization of *Toxoplasma gondii* from chickens and ducks from Egypt. Vet. Parasitol. 114: 89-95, 2003.
11. Puvanesuaran, V.R., Noordin, R. and Balakrishnan, V.: Isolation and genotyping of *Toxoplasma gondii* from free-range ducks in Malaysia. Avian Dis. 57: 128-132, 2013.
12. Yan, C., Yue, C.L., Yuan, Z.G., He, Y., Yin, C.C., Lin, R.Q., Dubey, J.P. and Zhu, X.Q.: *Toxoplasma gondii* infection in domestic ducks, free-range and caged chickens in southern China. Vet. Parasitol. 165: 337-340, 2009.
13. Cong, W., Huang, S.Y., Zhou, D.H., Xu, M.J., Wu, S.M., Yan, C., Zhao, Q., Song, H.Q. and Zhu, X.Q.: First report of *Toxoplasma gondii* infection in market-sold adult chickens, ducks and pigeons in northwest China. Parasite vectors. 5: 110, 2012.
14. Dubey, J.P. and Desmonts, G.: Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Vet. J. 19: 337-339, 1987.
15. Dubey, J.P.: Refinement of pepsin digestion method for isolation of *Toxoplasma gondii* from infected tissues. Vet. Parasitol. 74: 75-77, 1998.
16. Yan, C., Yue, C.L., Yuan, Z.G., Lin, R.Q., He, Y., Yin, C.C., Xu, M.J., Song, H.Q. and Zhu, X.Q.: Molecular and serological diagnosis of *Toxoplasma gondii* infection in experimentally infected chickens. Vet. Parasitol. 173: 179-183, 2010.
17. Sroka, J., Wojcik-Fatla, A., Szymanska, J., Dutkiewicz, J., Zajac, V. and Zwolinski, J.: The occurrence of *Toxoplasma gondii* infection in people and animals from rural environment of Lublin region – estimate of potential role of water as a source of infection. Ann. Agric. Environ. Med. 17: 125-132, 2010.
18. Murao, T., Omata, Y., Kano, R., Murata, S., Okada, T., Konnai, S., Asakawa, M., Ohashi, K. and Onuma, M.: Serological survey of *Toxoplasma gondii* in wild waterfowl in Chukotka, Kamchatka, Russia and Hokkaido, Japan. J. Parasitol. 94: 830-833, 2008.
19. Dubey, J.P., Edelhofer, R., Marcet, P., Vianna, M.C., Kwok, O.C. and Lehmann, T.: Genetic and biologic characteristics of *Toxoplasma gondii* infections in free-range chickens from Austria. Vet. Parasitol. 133: 299-306, 2005.
20. Dubey, J.P., Huong, L.T., Lawson, B.W., Subekti, D.T., Tassi, P., Cabaj, W., Sundar, N., Velmurugan, G.V., Kwok, O.C. and Su, C.: Seroprevalence and isolation of *Toxoplasma gondii* from free-range chickens in Ghana, Indonesia, Italy, Poland, and Vietnam. J. Parasitol. 94: 68-71, 2008.
21. Dubey, J.P., Marcet, P.L. and Lehmann, T.: Characterization of *Toxoplasma gondii* isolates in free-range chickens from Argentina. J. Parasitol. 91: 1335-1339, 2005.
22. Zhao, G.W., Wang, S., Hassan, I.A., Wang, W., Song, Z.H., Yang, L.W. and Yang, X.W.: Preliminary studies on the relevance of antibodies and bioassay to experimental infection with *Toxoplasma gondii* in ducks. Isr. J. Vet. Med. 70: 9-13, 2015.
23. Zia-Ali, N., Fazaeli, A., Khoramizadeh, M., Ajzenberg, D., Darde, M. and Keshavarz-Valian, H.: Isolation and molecular characterization of *Toxoplasma gondii* strains from different hosts in Iran. Parasitol. Res. 101: 111-115, 2007.