First Report of *Toxoplasma gondii* Seroprevalence in Pet Parrots in China

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Abstract

Toxoplasmosis, caused by the obligate intracellular protozoan parasite *Toxoplasma gondii*, has become a serious public health problem worldwide. *T. gondii* can infect almost all warm-blooded animals, including parrots. However, little is known of *T. gondii* infection in parrots in China. Antibodies against *T. gondii* in 311 parrots including 202 Budgerigars (*Melopsittacus undulatus*), 26 Lovebirds (*Agapornis* sp.), 22 Cockatiels (*Nymphicus hollandicus*), and 61 Alexandrine Parakeets (*Psittacula eupatria*) in the cities of Beijing and Weifang in north China were tested using the modified agglutination test (MAT). Twenty-six (8.36%) out of 311 serum samples were positive for *T. gondii* at the cutoff of 1:5. Among the four species, a higher seroprevalence of *T. gondii* was found in Cockatiels (13.64%, 95% confidence interval [CI] 0.00–27.98), although the difference was not statistically significant ($p=0.61$). Seropositivity rates against *T. gondii* in male parrots (10.43%, 95% CI 5.74–15.12) were not statistically different from that in female parrots (6.08%, 95% CI 2.23–9.93, $p=0.17$). The seropositivity of *T. gondii* in parrots from Weifang and Beijing was 11.11% (95% CI 6.13–16.09) and 5.70% (95% CI 2.08–9.31), respectively. The seroprevalence varied in parrots of different age groups, ranging from 5.71% (95% CI 1.27–10.15) to 13.00% (95% CI 6.41–19.69), however, the difference among age groups was not statistically significant ($p=0.12$). The seroprevalence of *T. gondii* infection in parrots in summer (11.63%, 95% CI 6.84–16.42) was significantly higher than in spring (4.32%, 95% CI 0.94–7.70, $p=0.02$). The results of the present survey indicated that parrots in China are exposed to *T. gondii*. To our knowledge, this is the first report of *T. gondii* seroprevalence in parrots in China.

Key Words: *Toxoplasma gondii*—Seroprevalence—Toxoplasmosis—Parrots—Modified agglutination test (MAT).

Introduction

TOXOPLASMA GONDII, AN OBLIGATE intracellular protozoan parasite, is responsible for toxoplasmosis in almost all warm-blooded animals worldwide, including humans and birds (Dubey 2010, Chen et al. 2012, Elmore et al. 2012, Li et al. 2012, Yuan et al. 2013). It is estimated that about one-third of the world population is chronically infected with *T. gondii* (Montoya et al. 2004, Zhou et al. 2008). The principal horizontal transmission of toxoplasmosis to humans is caused by ingestion of uncooked and raw meat contaminated with *T. gondii* tissue cysts or by consuming food or water contaminated with oocysts shed in the feces of infected cats (Dubey et al. 2008, Nowakowska et al. 2014).

China has a long history of raising parrots as pets. Many Chinese families own parrots because of their beautiful plumage and graceful posture and their ability to imitate sounds and repeat the words and sentences of humans. The parrot trade represents a lucrative business for breeders and pet shops, and the birds always sell at a high price. Parrots mainly originate from Beijing and Weifang in China, which breed nearly 70% of all parrots per year, and have become an important source of income for local people. Budgerigars (*Melopsittacus undulatus*), Lovebirds (*Agapornis* sp.), Cockatiels (*Nymphicus hollandicus*), and Alexandrine Parakeets (*Psittacula eupatria*) are high in the ranking of popular pet birds in China and are also widely represented in zoos and parks.

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Many surveys have reported the seroprevalence of *T. gondii* infection in several species of birds around the world (Dubey et al. 2010, Cabezón et al. 2011, Karatepe et al. 2011, Cong et al. 2012, Ferreira et al. 2012, Tian et al. 2012, Salant et al. 2013). However, no information regarding *T. gondii* infection in parrots in China is available. The objective of the present survey was to investigate the seroprevalence of *T. gondii* infection in Budgerigars, Lovebirds, Cockatiels, and Alexandrine Parakeets in China for the first time to provide baseline information for executing control strategies against *T. gondii* infection in parrots.

Materials and Methods

Ethics statement

This study was approved by the Animal Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences (approval no. LVRIAEC2012-008). The parrots from which blood was collected were handled in accordance with good animal practices required by the Animal Ethics Procedures and Guidelines of the People's Republic of China.

Naturally infected parrots

The examined parrots came from Beijing and Weifang in northern China. Beijing (39°26'–41°03'N, 115°25'–117°30'E) is the capital city of China with an average altitude of 43.5 meters. It has a typical temperate and monsoonal climate, with an annual precipitation of 626 mm and an average annual temperature of 12.6°C. Weifang is situated in the middle of Shandong Peninsula between east longitudes of 118°10' to 120°01' and north latitudes of 35°41' to 37°26'. The average altitude of Weifang city is 19.3 meters. The city has a north temperate and monsoonal climate, with an average annual temperature of 14.0°C and an annual precipitation of 615.3 mm.

Collection and preparation of serum samples

A total of 311 birds including 202 Budgerigars (*M. undulatus*), 26 Lovebirds (*Agapornis* sp.), 22 Cockatiels (*N. hollandicus*), and 61 Alexandrine Parakeets (*P. eupatria*) were sampled in spring and summer, 2013, in Beijing and Weifang from birds that had been bred in a semifree range system. The blood samples were obtained from the wing vein of parrots; serum samples were separated by centrifugation at 1000× g for 10 min and stored at –20°C until analysis. Data regarding species, geographic origin, age, and gender were obtained from local veterinary practitioners.

Serological examination

Serum samples were tested for antibodies to *T. gondii* using the modified agglutination test (MAT) as described previously (Cong et al. 2012, Wu et al. 2012, Yuan et al., 2013). MAT is known as a sensitive and specific technique for the detection of *T. gondii* antibodies and has been used in serological investigation of several animals worldwide (Cong et al. 2012, Wu et al. 2012, Alvarado-Esquivel et al. 2013, Siqueira et al. 2013, Yuan et al. 2013, Dubey et al. 2014). The test was considered positive when positive results were obtained at dilutions of 1:5 or higher. Antibody titers less than 1:5 were considered “suspect” and were retested. Positive and negative controls were included in each test.

Statistical analyses

Differences in the seroprevalence of *T. gondii*-infected parrots among different variables, including location, age, gender, season, and species, were analyzed using a chi-squared test by SAS (Statistical Analysis System, Version 8.0). Results were considered statistically significant when $p < 0.05$. These variables were also evaluated in the binary logit model as independent variables by forward stepwise regression analysis to test the seroprevalence (response variable) in the multivariable regression analysis. The best model was judged by the Fisher scoring algorithm. The effects could be included in the model when $p < 0.05$.

Results

A total of 26 (8.36%, 95% confidence interval [CI] 5.28–11.44) out of the 311 parrot serum samples were positive for *T. gondii* antibodies by MAT, with antibody titers of 1:5 in 14, 1:10 in 6, 1:20 in 2, 1:40 in 2, and 1:80 in two parrots (Table 1). Of these, positivity for antibodies to *T. gondii* varied from 3.85% (95% CI 0.00–11.24) to 13.64% (95% CI 0.00–27.98) in different species, with the highest seroprevalence being found in Cockatiels (13.64%, 95% CI 0.00–27.98, $p = 0.61$; Table 1).

No statistically significant difference was found in the seroprevalence of *T. gondii* between male parrots (10.43%, 95% CI 5.74–15.12) and female parrots (6.08%, 95% CI 2.23–9.93, $p = 0.17$). The prevalence in parrots of different age groups ranged from 5.71% (95% CI 1.27–10.15) to 13.00% (95% CI 6.41–19.69) (Table 2), but the difference was not statistically significant ($p = 0.12$). Parrots that were sampled in spring (4.32%, 95% CI 0.94–7.70) had a significantly higher seroprevalence of *T. gondii* than those

TABLE 1. SEROPREVALENCE OF *T. GONDII* INFECTION IN PARROTS IN BEIJING AND WEIFANG BY THE MODIFIED AGGLUTINATION TEST

Host	Antibody titers					No. negative	No. positive	% (95%CI)
	1:5	1:10	1:20	1:40	1:80			
Budgerigars (<i>Melopsittacus undulatus</i>)	9	5	1	2	1	184	18	8.91 (4.98–12.84)
Alexandrine parakeets (<i>Psittacula eupatria</i>)	3	1	0	0	0	57	4	6.56 (0.35–12.77)
Lovebirds (<i>Agapornis</i> spp.)	1	0	0	0	0	25	1	3.85 (0.00–11.24)
Cockatiels (<i>Nymphicus hollandicus</i>)	1	0	1	0	1	19	3	13.64 (0.00–27.98)
Total	14	6	2	2	2	285	26	8.36 (5.28–11.44)

CI, confidence interval.

TABLE 2. SEROPREVALENCE OF *T. GONDII* INFECTION IN PARROTS IN DIFFERENT REGIONS, GENDER, SPECIES, AGES, AND SEASONS

Variable	Category	No. tested	No. positive	% (95% CI)	p value
Region	Beijing	158	9	5.7 (2.08–9.31)	0.08
	Weifang	153	17	11.11 (6.13–16.09)	
Gender	Male	163	17	10.43 (5.74–15.12)	0.17
	Female	148	9	6.08 (2.23–9.93)	
Species	Budgerigars (<i>Melopsittacus undulatus</i>)	202	18	8.91 (4.98–12.84)	0.61
	Alexandrine parakeets (<i>Psittacula eupatria</i>)	61	4	6.56 (0.35–12.77)	
	Lovebirds (<i>Agapornis</i> sp.)	26	1	3.85 (0.00–11.24)	
	Cockatiels (<i>Nymphicus hollandicus</i>)	22	3	13.64 (0.00–27.98)	
Age	≤5 months	105	6	5.71 (1.27–10.15)	0.12
	6–12 months	100	13	13.00 (6.41–19.69)	
	13–18 months	106	7	6.60 (1.88–11.33)	
Season	Spring	139	6	4.32 (0.94–7.70)	0.02
	Summer	172	20	11.63 (6.84–16.42)	
Total		311	26	8.36 (5.28–11.44)	

CI, confidence interval.

sampled in summer (11.63%, 95% CI 6.84–16.42, $p=0.02$). Statistical analysis of the originating regions showed that parrots from Weifang city had a higher *T. gondii* seropositivity (11.11%, 95% CI 6.13–16.09) than the birds from Beijing (5.70%, 95% CI 2.08–9.31), but the difference was not statistically significant ($p=0.08$). In the multivariable regression analysis, no risk factors could be included in the final model because all the p values were above 0.05.

Discussion

Parrots are the most popular pet birds in China. However, the role of pet birds in the transmission of *T. gondii* should be given more attention because they can serve as sources for *T. gondii* infection in cats (Ruiz et al. 1980, Dubey et al. 2002), especially in some rural areas of China. In the present study, we investigated the seroprevalence of *T. gondii* in parrots from two main breeding places in China—the cities of Beijing and Weifang.

The overall seroprevalence of *T. gondii* infection in parrots examined in the present study was 8.36% (95% CI 5.28–11.44), which was lower than that of wild birds in Israel (Salant et al. 2013), Spain (Cabezón et al. 2011), Colorado (Dubey et al. 2010), peafowls in Yunnan Province, China (Tian et al. 2012), and pigeons in Lanzhou, China (Cong et al. 2012), but higher than that of pigeons in Turkey (0.93%) (Karatepe et al. 2011). The difference in *T. gondii* seropositivity in birds may be related to differences in their living environments, immune capacities, serologic tests used, feeding conditions, resistance to the pathogen, as well as animal husbandry practices and animal welfares.

Old animals have more opportunities to acquire *T. gondii* infection by ingesting food contaminated by oocysts. So, in many other studies, *T. gondii* seroprevalence rates are usually higher in older animals than in young ones (Alvarado-Esquivel et al. 2012, Wang et al. 2012). In the present study, however, age was not shown as an important risk factor for *T. gondii* infection in parrots, although a higher sero-

prevalence was found in the age group of 6 and 12 months (13.00%, 95% CI 6.41–19.69, $p=0.12$).

In China, most parrots are bred in a semi-free-range system, and the birds have relatively similar opportunities to contact food or water contaminated with *T. gondii* when they gather together. Thus, there was no statistically significant difference in the seroprevalence between male parrots (10.43%, 95% CI 5.74–15.12) and females (6.08%, 95% CI 2.23–9.93), which is different from previous reports that the females are more susceptible than males (Roberts et al. 1995, Severance et al. 2012, Minbaeva et al. 2013).

Previous studies have shown that Budgerigars are relatively resistant to clinical toxoplasmosis (Dubey et al. 2002), while little is known of *T. gondii* infection in other species of Psittaciformes. With the exception of Budgerigars, the present study was the first to investigate *T. gondii* seropositivity in Lovebirds, Cockatiels, and Alexandrine Parakeets in China, and worldwide, as far as we know. Among these four species of parrots, a higher seroprevalence was detected in Cockatiels (13.63%, 95% CI 0.00–27.98) and a lower prevalence was in Lovebirds (3.85%, 95% CI 0.00–11.24), but the difference was not statistically significant ($p=0.61$).

Beijing and Weifang are located in north China, and their climates are typically high temperature and rainy in summer and dry and strong winds in spring. Statistical analysis showed a significant difference in *T. gondii* seroprevalence of birds collected between spring and summer ($p=0.02$). The moist conditions in summer can increase oocyst survival during long periods of heat, and thus allow more opportunities for contact of the oocysts with other animals, which may also pose a risk for human infection. The seropositivity of *T. gondii* was not statistically different between Beijing and Weifang ($p=0.08$), which have similar climates. On the basis of the forward stepwise regression analysis, no factors fit to the model; this may be because these variables did not contribute to the *T. gondii* prevalence in parrots.

Parrots produced in Beijing and Weifang are traded throughout China every year, which may serve as a vehicle for zoonotic transfer. Generally, *T. gondii* infection in parrots is not harmful for humans. However, dead parrots both from parks and from households where they have been kept as pets are often discarded without any biosafety considerations. The dead birds may be eaten by cats, and, if the birds contained *T. gondii*, the cats may become infected with the parasite and subsequently shed oocysts. Ingestion of water, vegetables, fruits, and other food contaminated with *T. gondii* oocysts is considered one of the important routes of toxoplasmosis transmission to humans (Nowakowska et al. 2014). Thus, future surveys of *T. gondii* infection should include parrots.

Conclusion

The present survey revealed the existence of *T. gondii* infection in four species of parrots (Budgerigars, Lovebirds, Cockatiels, and Alexandrine Parakeets) in Beijing and Weifang in north China, which raised the need for attention from parrot breeders, workers in pet shops and markets, tourists, zoo veterinarians, and governmental authorities in view of the public health concerns. To our knowledge, the present study is the first to document the *T. gondii* seroprevalence in parrots in China.

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