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Short Communication

Genetic Characterization of *Toxoplasma gondii* from Zoo Wildlife and Pet Birds in Fujian, China

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Abstract

Background: Toxoplasmosis, a worldwide zoonotic disease, is caused by *Toxoplasma gondii*. The distribution of genetic diversity of *T. gondii* in wild animals is of great importance to understand the transmission of the parasite in the environment. However, little is known about *T. gondii* prevalence in wild animals and birds in China.

Methods: We conducted the genetic characterization of *T. gondii* isolated from Zoo Wild Animals and Pet Birds in Fujian Province, Southeastern China. Heart tissues were collected from 45 zoo animals and 140 pet birds. After identified using B1 gene, the genetic diversity of *T. gondii* isolates were typed at 11 genetic markers, including SAG1, 5' and 3'-SAG2, alternative SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, Apico, and CS3.

Results: Seven of 45 zoo animals and 3 of 140 pet birds were positive by PCR amplification using *T. gondii* B1 gene specific primers. Of these positive isolates, 3 isolates from Black-capped (*Cebus apella*), Peacock (*Peafowl*) and Budgerigar (*Melopsittacus undulatus*) were successfully genotyped at 11 genetic loci, and grouped to three distinct genotypes: ToxoDB Genotype #9, #2 and #10, respectively.

Conclusion: This is the first genotyping of *T. gondii* isolated from zoo wild animals and pet birds in Fujian, China. There is a potential risk for the transmission of this parasite through zoo wild animals and pet birds in this region.

Introduction

The successful pathogen, *Toxoplasma gondii*, is able to infect virtually all warm-blooded animals including birds and mammals in worldwide, obligating to the zoonotic toxoplasmosis (1). Human beings acquired the disease mainly through ingestion of tissue cysts or oocysts of *T. gondii*, containing in food or water (2). *T. gondii* infection in healthy adults rarely causes clinical symptoms, but can lead to be fatal in immunocompromised individuals (2-4).

In wildlife, many animals also were infected by *T. gondii*. Some studies reported high infection rate of *T. gondii* in zoo animals and wild birds. For example, Murata in early 1989 just showed 29.28% (53/181) and 16.94% (61/179) of *T. gondii* IgG in mammals and wild birds, respectively, by serological survey (5). Zhang et al. also reported there had 25% (4/16) of *T. gondii* antibody in primates, 69.4% (25/36) in carnivores, 27.6% (8/29) in herbivores and 11.11% (4/36) in birds in China (6). There was 36.17% (51/141) in felids and 10.81% (4/37) in prosimians (7). Other recent reports also showed the high infection rate such as 36.1% (73/202) of wild birds captured from the wild environment (8) and 12.46% (39 /313) of house sparrows in China (9).

In recent years, different sources of *T. gondii* strains were identified using multilocus polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, which was established for genotyping by Su and other scholars (10-14). The distribution of genetic diversity of *T. gondii* in wildlife animals is of great importance to understand the transmission of this parasite in the environment. Some studies have identified several genetic characterizations of *T. gondii* isolates from cats, bats, sheep, and birds in China (15-18). However, little information concerning *T. gondii* isolates from zoo animals and pet birds in southeast China.

The objectives of the present study were to determine the prevalence and genetically characterize *T. gondii* in wild animals from Fuzhou zoo and pet birds' farms in Fujian province, southeastern China.

Materials and Methods

Sample collection

A total of 185 animals were randomly collected from Fujian province during 2012 and 2014. The animals were consisted of 24 species, listing in Table 1. The 45 heart tissue samples from were collected from zoo animals, and 140 heart tissue samples were collected from pet birds. All tissue samples were stored at -20 °C prior to use.

Genomic DNA extraction

Genomic DNA was extracted from heart tissue samples of wild animals and pet birds using TIANamp Genomic DNA kit (TianGen™, Beijing, China). In brief, 30 mg of heart tissue were treated with sodium dodecyl sulphate/proteinase K at 56 °C for overnight digestion in a thermostat water bath. DNA samples were prepared according to the previous study (19) and eluted into 60 uL with elution buffer.

The positive genomic DNA of *T. gondii* RH (Type I) and PRU (Type II) strains were kindly provided by Fujian Normal University and Lanzhou Veterinary Research Institute, respectively.

Genetic characterization of T. gondii isolates

At first, all the DNA samples from tissues of wild animals and pet birds were identified using semi-nested PCR method based on *T. gondii* B1 gene as described previously (20). In brief, the first round were amplified at 35 cycles with 93 °C for 10 s, 57 °C for 10 s and 72 °C for 30 s. Then the nested PCR was beginning at 93 °C 10 s for denaturation, following by annealing at 62.5 °C for 10 s and the 72 °C 10 s for extension (21). The PCR amplification was performed by Veriti™ 96-

Well Thermal Cycler (ABI), and the products were separated by 1.5% agarose gel, visualized under ultraviolet light.

The positive samples were then genotyped using Multilocus PCR-RFLP method as previously described by amplifications of 11 markers, including SAG1, 5' and 3'-SAG2, alternative SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, Apico, and CS3 (13, 15, 22).

The reaction volume (25 µL) contained 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.3 mM each primer, 0. 25 mM each dNTP, 1.25 U Golden DNA polymerase (Tiangen, Beijing, China), and 2 µL *T. gondii* DNA. All samples were incubated at 95 °C for 5 min, then 35 cycles of PCR at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 40 s and 72 °C for 7 min. Similar program was used for the nested PCR. The nested PCR was carried out with an annealing temperature at 60 °C for 60 s for all the markers except Apico, which was amplified at 55 °C. The PCR products were digested using restriction enzymes and separated by 2.5% agarose gel. The bands were observed under ultraviolet light. The previous studies (9, 13, 14, 15) are used to compare with the results of the genetic characterization of the Chinese *T. gondii* in our present work.

Results

Of the total 185 samples, 10 samples were detected to be positive for the *T. gondii* B1 gene, including 7 zoo animals (1 Black-capped, 1 Wild Red Dog, 1 Lemur, 1 zebra, 1 Red panda, 1 Peacock, 1 Red-crowned Crane, 15.56%, 7/45) and 3 pet birds (1 Budgerigar, 1 Black-billed Magpie, 1 Zebra Finch, 2.14%, 3/140,) (Table 1).

Table 1: Animals from Fujian province, China used in the present study and the prevalence of *T. gondii* infection in these animals

Class	Animal	Number	Positive
Zoo animals	Himalayan Black Bear (*Ursus thibetanus*)	2	0
	Wild Red Dog (*Cuon alpinus*)	2	1
	Masked palm civet (*Paguma larvata taivana*)	1	0
	Lemur (*Aye-Aye*)	3	1
	Patas monkey *(Erythrocebus patas*)	1	0
	Squirrel Monkey (*Saimiri sciureus*)	3	0
	Black-capped (*Cebus apella*)	1	1
	Francois's Leaf Monkey (*Trachypithecus francoisi*)	1	0
	Barking Deer (*Muntiacus muntjak*)	6	0
	Zebra (*Equus burchellii*)	1	1
	Big antelope (*T.derbianus*)	1	0
	Alpaca (*Lama pacos*)	1	0
	Red panda (*Ailurus fulgens*)	10	1
	Black Swan (*Cygnus atratus*)	4	0
	Brown Hawk-owl (*Ninox scutulata*)	1	0
	Peacock (*Peafowl*)	5	1
	Red-crowned Crane (*Grus japonensis*)	1	1
	Grey-faced Buzzard (*Butastur indicus*)	1	0
	Total	45	7
Pet birds	Black-billed Magpie (*Pica pica*)	18	1
	Budgerigar (*Melopsittacus undulatus*)	67	1
	Fischer's Lovebird (*Agapornis personata fische*)	28	0
	Zebra Finch (*Poephila guttata*)	20	1
	Cockatiel (*Nymphicus hollandicus*)	3	0
	Starling (*Acridotheres cristatellus*)	4	0
	Total	140	3

The positive rate of *T. gondii* DNA in zoo animals was significantly higher than that of pet birds in Fujian, China ($P<0.05$. Further genotyped the positive samples using Multi-locus PCR-RFLP method showed that 3 distinct genotypes (ToxoDB Genotype #9, #2 and #10) of *T. gondii* from Black-capped (*Cebus apella*), Peacock (*Peafowl*) and Budgerigar (*Melopsittacus undulatus*) respectively were identified (Table 2).

Table 2: Results of genotyping of *T. gondii* from Black-capped, Peacock and Budgerigar

Isolate	Host	SAG 1	5+3 SAG2	SAG 2	SAG 3	BTU B	GRA6	C22-8	C29-2	L358	PK1	Apico	CS3	Genetype
RH	Human	I	I	I	I	I	I	I	I	I	I	I	I	Type I ToxoDB#10
PRU	Human	II or III	II	II	nd	II	II	II	II	II	II	I	II	Type II ToxoDB#1
TgBcFZ	Black-capped	u-1	II	II	III	III	II	II	III	II	II	I	II	ToxoDB#9
TgCpFZ	Peacock	II or III	III	III	III	III	III	III	III	III	III	III	III	Type III ToxoDB#2
TgBFZ	Budgerigar	I	I	I	I	I	I	I	I	I	nd	I	I	Type I ToxoDB#10

nd: represents no data, u-1: represents unique RFLP genotypes

Discussion

By using sequence-specific primers, the relative number of copies of a particular DNA sequence can be determined. There are many applications of this technique in the field of diagnosis (23-25). *T. gondii* can be detected by semi-nested polymerase chain reaction (PCR) using B1 genes in pig, cat, and other wild animals (26-29). In the present study, we identified the *T. gondii* infection in Black-capped, Wild Red Dog, Lemur, zebra, Red panda, Peacock, Red-crowned Crane, Budgerigar, Black-billed Magpie, Zebra Finch in China, which indicated these animals could be served as a potential source of infection for other animals and even humans.

PCR-RFLP method is frequently used to identify differences between homologous DNA sequences (13,15), which have been successfully used for genotyping of *T. gondii* from cat, chicken, human, cougar, sheep, birds, sparrow, pig and other animals (2, 9, 13, 15-18, 26). Further genotyped the positive samples using Multi-locus PCR-RFLP method showed that 3 distinct genotypes (ToxoDB Genotype #9, #2 and #10) of *T. gondii* from Black-capped (*Cebus apella*), Peacock (*Peafowl*) and Budgerigar (*Melopsittacus undulatus*) respectively were identified (Table 2). Zoo animals and pet birds have formed a huge industry in order to prevent rare wild animals from extinction and to boost the economy. Fujian province has a great advantage in convenient transportation because of its southeast coastal location in China, so the exchange of wild animals and pet birds becomes more ordinary. The results in this present study will enhance our understanding of the epidemiology and prevention of *T. gondii* in zoo wild animals and pet birds.

One sample isolated from Budgerigar belonged to ToxoDB Genotype #10, supporting the previous study that ToxoDB Genotype #10 had been isolated from 3 sparrows in Fuzhou (18). One sample isolated from Black-capped belonged to ToxoDB Genotype #9. This result indicated that ToxoDB Genotype #9 was predominant not only in southern, southwestern and central parts of China but also in southeast part of China (15, 17, 30-32). In addition, our finding support that ToxoDB Genotype #9 is at CS3 locus. One sample isolated from Common Peafowl belonged to ToxoDB Type III Genotype #2. A few Type

III genotypes were detected (13, 33). This is the first finding, revealing that Type III variant occurs in China. Our study is also the first report of genetic typing of *T. gondii* from zoo wild animals and pet birds in china. The high genetic diversity of *T. gondii* genotypes in Fujian may cause by the high number of cats in city and other feline in mountainous areas.

Conclusion

The positive rate of *T. gondii* DNA was high in zoo animals (7/45, 15.56%) but very low in birds (3/140, 2.14%) in Fujian, China. Further genotyping analysis confirmed that the presence of *T. gondii* in zoo wildlife and pet birds were ToxoDB Genotype #10, Genotype #9 and Genotype #2, which suggested the integrated measures should be taken to prevent *T. gondii* infection in humans or other animals.

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