

Genetic characterization of *Toxoplasma gondii* from domestic animals in central China

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Abstract. *Toxoplasma gondii* is an obligate intracellular parasite that has a remarkable ability to infect almost all warm-blooded animals, including humans. This study was aimed to determine the genetic characteristics of *T. gondii* isolates from domestic animals in Henan Province, central China. A total of 363 DNA samples, including 208 from hilar lymph nodes of pigs, 36 from blood samples of cats, 12 from tissues of aborted bovine fetuses and 107 from blood samples of dams with history of abortion in Henan Province, were examined for the presence of *T. gondii* by nested PCR based on B1 gene. The positive DNA samples were further genotyped by PCR-RFLP at 11 markers, including SAG1, (3'+ 5') SAG2, alt.SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico. DNA samples from 9 pigs, 5 cats, and 4 dairy cows were *T. gondii* B1 gene positive. Nine samples were successfully genotyped at all genetic loci, of which 5 samples from pigs, and 2 from cats were identified as ToxoDB genotype #9, and 2 samples from cows belonged to ToxoDB genotype #225. To our knowledge, the present study is the second report of genetic typing of *T. gondii* isolates from cattle in China, and the first report of *T. gondii* ToxoDB#225 from cattle.

INTRODUCTION

Toxoplasma gondii infections are widely prevalent in warm-blooded vertebrates, including mammals and birds worldwide. *T. gondii* antibodies have been found in one third of the world's human population (Dubey, 2010). Humans become infected by consuming food or water contaminated with oocysts, ingesting tissue cysts from undercooked meat, or transplacental transmission of tachyzoites from mother to the foetus (Dubey, 2010). Commonly, primary infections in immunocompetent adults are asymptomatic but in immunocompromised patients it may cause life-threatening encephalitis due to the reactivation of latent infection (Montoya & Liesenfeld, 2004).

Toxoplasma gondii has subpopulation structures in different geographical regions. Most *T. gondii* isolates from human and animals in North America, Europe and Africa

have been grouped into one of three clonal lineages (Type I, II and III) (Su *et al.*, 2010). And the fourth clonal lineage (type 12) has been described and is the most common type in wildlife in North America. In contrast, *T. gondii* isolates in South America are diverse (Dubey *et al.*, 2011; Khan *et al.*, 2011).

In China, with rich animal biodiversity, thirteen genotypes of *T. gondii* have been identified by PCR-RFLP from animals and humans (Wang *et al.*, 2013a; Zhang *et al.*, 2013; Ge *et al.*, 2014). However, most genetic information was from *T. gondii* in cats and pigs (Qian *et al.*, 2012; Wang *et al.*, 2013a; Tian *et al.*, 2014). It is necessary to genotype more isolates of *T. gondii* from different animals and regions to better understand the genetic characteristics of *T. gondii* in China. So far, there is only limited genetic information of *T. gondii* from pigs in Henan Province, central China (Zhou *et al.*, 2010). The objective of this study was to determine

the genetic characteristics of *T. gondii* in domestic animals in Henan Province, central China.

MATERIALS AND METHODS

A total of 208 pig hilar lymph nodes were collected between August 2012 and September 2013 in Luoyang, Nanyang and Pingdingshan, Henan Province. Of which, 117 hilar lymph nodes were collected from slaughter pigs, which were all raised in semi-intensive systems and mainly transported to market for public. Another 91 post-mortem hilar lymph nodes were collected from sick pigs after they were admitted to the veterinary hospital of Henan University of science and technology. And these pigs were free-range or raised in semi-intensive systems.

Venous blood samples were collected by local veterinary practitioners from 36 pet cats in Luoyang and Nanyang between August 2012 and July 2013. All the animal owners were asked for details of the animals breed, age, sex, and living conditions using a structured questionnaire.

107 blood samples of dams with history of abortion, and of which twelve aborted fetuses of Holstein cows were collected from 7 dairy herds in Luoyang and Pingdingshan between March and November 2013. Tissue samples of brain, heart, lung, liver, spleen and kidney of the fetuses were stored at -20°C until use.

Genomic DNA was extracted from blood and tissue samples of different animals using the Universal Genomic DNA Kit (Beijing Zoman Biotechnology Co., Ltd) according to the manufacturer's recommendations. A nested PCR targeting the *T. gondii* B1 gene was performed to detect possible infection with *T. gondii*. DNA samples giving positive B1 amplification were then used for genetic characterization.

Genotyping of *T. gondii* isolates was carried out using a previously described 11 Markers PCR-RFLP method (Su *et al.*, 2010). Individual DNA sample was amplified with nested PCR based on 11 genetic markers, including SAG1, (3'+5') SAG2, alt.SAG2, SAG3, BTUB, GRA6, L358, PK1, c22-8, c29-2

and Apico. The nested PCR products were digested by restriction enzymes, and resolved in agarose gel by electrophoresis to reveal the RFLP patterns according to Su *et al.* (2010). Allele types for all isolates were determined based on the RFLP patterns of four reference strains including GT1, PTG, CTG, and MAS (Su *et al.*, 2010).

RESULTS AND DISCUSSION

Toxoplasma gondii DNA was detected in all three species domestic animals by nested PCR based on B1 gene. Nine out of 208 (4.3%) pig hilar lymph node DNA samples were positive, with 2 samples from slaughter pigs and 7 from sick pigs. The positive rate of *T. gondii* in sick pigs (7.7%, 7/91) was higher than the slaughter pigs (1.7%, 2/117). The positive rate of *T. gondii* in pet cats was 13.9% (5/36). Of 107 dairy cow blood samples, 3.7% (4/107) were PCR amplification positive, however, no *T. gondii* specific DNA was detected from all the tissues of 12 aborted fetuses. The prevalences of *T. gondii* infection in pigs, cats, and cattle in Henan Province in this study were lower than the seroprevalences reported in other regions of China, 12.0% ~ 53.4% in pigs (Wang *et al.*, 2013a), 25.1% ~ 57.8% in cats (Qian *et al.*, 2012; Tian *et al.*, 2014), and 5.7% ~ 12.8% in cattle (Zhou *et al.*, 2012; Ge *et al.*, 2014), this differences may be due to different detection methods used.

Due to low DNA concentration, only 9 B1 gene positive DNA samples (5 from pigs, 2 from cats and 2 from cows) gave complete genotyping results at all 11 gene loci. Two genotypes were identified, including ToxoDB#9 and ToxoDB#225. 5 samples from pigs and 2 from pet cats were identified as ToxoDB#9, and 2 samples from dams were identified as ToxoDB#225 (Table 1).

Genotype ToxoDB#9 is a predominant lineage prevalent in Mainland China. This genotype has been previously found in four animal species; in cats in Beijing, Guangdong, Hubei, Anhui, Guizhou, and Yunnan Province (Qian *et al.*, 2012; Wang *et al.*, 2013b; Tian *et al.*, 2014); in pigs in Guangdong, Yunnan, Anhui, Jiangxi, and

Table 1. Multilocus genotyping of *Toxoplasma gondii* from domestic animals in Henan Province, central China

Isolate ID	Host	Location	SAG1	5+3'SAG2	all_SAG2	SAG3	BTUB	GRA6	C22-8	C29-2	L358	PK1	Apico	Genotype
GT1, reference	Goat	USA	I	I	I	I	I	I	I	I	I	I	I	Type I, ToxoDB #10
PTG, reference	Sheep	USA	II/III	II	II	II	II	II	II	II	II	II	II	Type II, ToxoDB #1
CTG, reference	Cat	USA	II/III	III	III	III	III	III	III	III	III	III	III	Type III, ToxoDB #2
MAS, reference	Human	France	u-1 ^a	I	II	III	III	III	u-1 ^a	I	I	III	I	ToxoDB #17
TgPHN1	Pig	Pingdingshan, Henan	u-1 ^a	II	II	III	III	II	II	III	II	II	I	ToxoDB #9
TgPHN2	Pig	Luoyang, Henan	u-1 ^a	II	II	III	III	II	II	III	II	II	I	ToxoDB #9
TgPHN3	Pig	Luoyang, Henan	u-1 ^a	II	II	III	III	II	II	III	II	II	I	ToxoDB #9
TgPHN4	Pig	Luoyang, Henan	u-1 ^a	II	II	III	III	II	II	III	II	II	I	ToxoDB #9
TgPHN5	Pig	Luoyang, Henan	u-1 ^a	II	II	III	III	II	II	III	II	II	I	ToxoDB #9
TgCatHN1	Cat	Luoyang, Henan	u-1 ^a	II	II	III	III	II	II	III	II	II	I	ToxoDB #9
TgCatHN2	Cat	Nanyang, Henan	u-1 ^a	II	II	III	III	II	II	III	II	II	I	ToxoDB #9
TgCowHN1	Cow	Luoyang, Henan	I	I	I	III	I	I	I	I	I	I	I	ToxoDB #225
TgCowHN2	Cow	Luoyang, Henan	I	I	I	III	I	I	I	I	I	I	I	ToxoDB #225

^a u-1 represent unique RFLP genotypes.

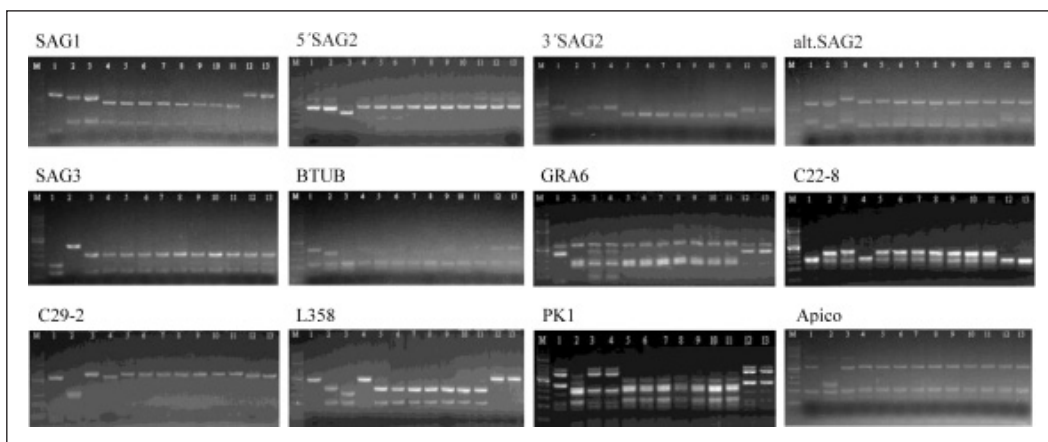


Figure 1. PCR-RFLP analysis of *Toxoplasma gondii* isolates from domestic animals in Henan Province, central China. M: 50 bp plus DNA marker; Lanes 1-13 represent GT1, PTG, CTG, MAS, TgPHN1, TgPHN2, TgPHN3, TgPHN4, TgPHN5, TgCatHN1, TgCatHN2, TgCowHN1 and TgCowHN2, respectively.

Henan Province (Jiang *et al.*, 2013); in voles in Hubei and Jilin Province (Wang *et al.*, 2013a; Zhang *et al.*, 2014); in bats in Guangxi Province (Jiang *et al.*, 2014). It is the first time to genotyping *T. gondii* isolates from cats in Henan Province, central China. Worldwide, ToxoDB#9 has been isolated in other Asian countries, such as Sri Lanka, Vietnam (Dubey *et al.*, 2007b, 2007c), as well as North and South America (Dubey *et al.*, 2007a, 2008).

Limited genetic information was available about this parasite in cattle in China. There was only one report about *T. gondii* genotyping from cattle in China, and type I variant (type III at GRA6 locus and type I at the other 10 loci) was identified in Jilin Province (Ge *et al.*, 2014). In this study, genotype ToxoDB#225 (type III at SAG3 locus and type I at the other 10 loci) was identified from 2 dams, which has been previously reported in chickens in Anhui Province and cats in Yunnan Province, China (Wang *et al.*, 2013a; Tian *et al.*, 2014). It was the first time to report ToxoDB#225 in cattle in China. Further studies on more samples collected from different regions are needed to understand the genetic diversities of *T. gondii* from cattle in China.

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