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ORIGINAL PAPER

Toxoplasma gondii, *Dirofilaria immitis*, feline immunodeficiency virus (FIV), and feline leukemia virus (FeLV) infections in stray and pet cats (*Felis catus*) in northwest China: co-infections and risk factors

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Abstract This study was conducted to estimate the prevalence of *Toxoplasma gondii*, *Dirofilaria immitis*, feline immunodeficiency virus (FIV), and feline leukemia virus (FeLV) infections among stray and pet cats in Lanzhou, northwest China, and to identify the influence of age, gender, and regions on seropositivity. *T. gondii* antibodies were examined in cat sera by the modified agglutination test (MAT). The circulating antigens of *D. immitis* and FeLV and specific antibodies to FIV were examined using kits commercially available. The overall prevalence of *T. gondii*, FIV, FeLV, and *D. immitis* was 19.34, 9.12, 11.33, and 3.04 %, respectively. For the genetic characterization of *T. gondii* genotypes in cats, genomic DNA was extracted from the seropositive cats and the *T. gondii* B1 gene was amplified using a semi-nested PCR. DNA samples giving positive B1 amplification were then genotyped using multilocus PCR-RFLP. Two *T. gondii* genotypes (ToxoDB#9 and ToxoDB#1) were identified. Results of the multivariate logistic regression analysis showed that older cats are more likely to be seropositive than juveniles for *T. gondii*, FIV, FeLV, and *D. immitis*. This is the first report of *T. gondii* genotypes in cats in northwest China. Moreover, the present study is the first study of retrovirus and *D. immitis* seroprevalence in cats in China. The results revealed that *T. gondii*, FIV, and FeLV infections are common in stray and pet cats in northwest China.

Keywords *Toxoplasma gondii* · *Dirofilaria immitis* · Feline immunodeficiency virus · Feline leukemia virus · Cats · Northwest China

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Introduction

Toxoplasma gondii, heartworm (*Dirofilaria immitis*), feline leukemia virus (FeLV), and feline immunodeficiency virus (FIV) are important infectious pathogens of cats. *T. gondii* is a zoonotic parasite that infects humans and other animals worldwide (Dubey 2010), and cats are the crucial species in the epidemiology of *T. gondii* infection, as they are the definitive host of the parasite that can directly excrete resistant oocysts in the environment (Dubey 2010). Usually, *T. gondii* infections in cats are typically asymptomatic, but the infection in other mammals and birds can induce severe diseases and even death (Elmore et al. 2010; Ramos et al. 2011). Although

canids are a final host for *D. immitis*, its life cycle can also be completed in cats (Nelson 2008). Many cats suffer from heartworm infection well, and in some, the disease is cured without intervention and without a fatal result. However, some infected cats undergo recurrent signs and symptoms of infection such as chronic cough, respiratory stress, and asthma (Litster and Atwell 2008; Bowman and Atkins 2009; Park et al. 2014). FIV is associated to HIV, which can induce immunosuppression in some cats, according to the phase of infection. FeLV is associated to human T lymphocytic virus and can also cause immunosuppression in cats (Little 2011; Tiao et al. 2013). FeLV and FIV are two common and important retroviral pathogens of cats all over the world (Dunham and Graham 2008). Diseases associated with FeLV and FIV may affect any organ, including lymphoma, blood dyscrasias, central nervous system and eye diseases, gingivostomatitis, and secondary and opportunistic infections (Little 2011). Previous studies of cat serum have demonstrated an association between seropositivity for *T. gondii* and these two viral pathogens in some countries (Dorny et al. 2002; Maruyama et al. 2003; Dubey et al. 2009).

With the rapid economic development in the last three decades, the number of cats is increasing rapidly in China, but new problems arise because cats can transmit a number of zoonotic pathogens, including parasites (Gerhold and Jessup 2013). Epidemiological data are necessary to define preventive, management, and therapeutical measures for stray, feral, and domestic cats. Although several studies have reported the *T. gondii* prevalence in cats in various regions in China (Dubey et al. 2007; Chen et al. 2011; Qian et al. 2012; Wang et al. 2013; Tian et al. 2014), including Lanzhou (Wu et al. 2011), little is known of the genotypes of *T. gondii* in cats in northwest China. Here, we investigated the genotypes of *T. gondii* in cats in northwest China for the first time. Moreover, we also investigated the prevalence of these four pathogens in cats in northwest China and examined the potential association of infection with these pathogens in the same host and the possible risk factors that might be associated with infection with these pathogens.

Materials and method

Naturally infected cats

This study was approved by the Animal Ethics Committee of Jilin Agricultural University. Between March 2014 and May 2015, a total of 362 blood samples were obtained from stray and pet cats in Lanzhou, Dingxi, and Wuwei cities of Gansu province. Information regarding the age, gender, and geographical origin of pet cats was obtained from their owners, and the biometric data of stray cats were estimated based on body condition and dental age (Table 1). Blood samples were kept at room temperature for 2 h and centrifuged at 3000 rpm for 5 min, and the separated serum samples were stored at −20 °C until further analysis.

Serological examination

Antibodies to *T. gondii* were determined in cat sera by the modified agglutination test (MAT) as described previously (Wu et al. 2011). In brief, sera were added to "U"-bottom 96-well microtiter plates, diluted twofold starting from 1:25 to 1:1600; the plates were shaken for 2 min and then incubated at 37 °C overnight without shaking. Sera with MAT titers of 1:25 or higher were considered positive, and those sera with dubious results were re-tested. Positive and negative controls were incorporated in each test.

The assay utilized to detect the circulating antigens of *D. immitis* and FeLV and specific antibodies to FIV was the SNAP® Feline Triple® Test (IDEXX Laboratories, Westbrook, ME, USA). The assays were performed as per the manufacturer's instructions.

Genetic characterization of *T. gondii*

The brain, heart, and lung of seropositive cats were used for DNA extraction. Genomic DNA was extracted from these tissues using TIANamp Genomic DNA kit (TianGen™, Beijing, China) according to the manufacturer's recommendations. Then, a semi-nested PCR targeting the *T. gondii* B1 gene was performed to detect possible infection with *T. gondii* (Hill et al. 2006). DNA samples giving positive B1 amplification were then used for genetic characterization. Genotyping was conducted using 11 genetic markers for PCR-RFLP (i.e., SAG1, SAG2, alter.SAG2, SAG3, BTUB, GRA6, c22-8, L358, c29-2, PK1, and Apico) according to previously reported protocol (Yu et al. 2013; Qin et al. 2014; Cong et al. 2015; Samico-Fernandes et al. 2015). Nine reference *T. gondii* strains were included as the positive controls including GT1, PTG, CTG, MAS, TgCgCa1, TgCatBr5, TgWtdSc40, TgCatBr64, and TgToucan (Table 3). The nested PCR products were digested with restriction enzymes for 1 h, and the temperature for each enzyme was used according to the instructions for each enzyme. The restriction fragments were resolved in 2.5–3 % agarose gel, stained by the GoldenView™, and photographed using a gel documentation system (UVP GelDoc-ItTM Imaging System, Cambridge, UK).

Statistical analysis

Differences in the seroprevalence among different variables including location, age, gender, and types were analyzed using

Table 1 Seroprevalence of feline immunodeficiency virus (FIV), feline leukemia virus (FeLV), *Toxoplasma gondii*, and *Dirofilaria immitis* in stray and pet cats in northwest China

Characteristics	Cat tested	Feline immunodeficiency virus		Feline leukemia virus		*Toxoplasma gondii*		*Dirofilaria immitis*	
	No.	No. positive	%	No. positive	%	No. positive	%	No. positive	%
Age (months)									
<6	147	6	4.08	14	9.52	22	14.97	1	0.68
6–12	92	9	9.78	11	11.96	19	20.65	4	4.35
13–18	79	8	10.13	10	12.66	14	17.72	3	3.80
>18	44	10	22.73	6	13.64	15	34.09	3	6.82
Gender									
Male	211	19	9.00	25	11.85	38	18.01	7	3.32
Female	151	14	9.27	16	10.60	32	21.19	4	2.65
Types									
Stray cats	260	27	10.38	31	11.92	59	22.69	9	3.46
Pet cats	102	6	5.88	10	9.80	11	10.78	2	1.96
Region									
Lanzhou	140	14	10.00	17	12.14	27	19.29	5	3.57
Dingxi	121	8	6.61	12	9.92	25	20.66	4	3.31
Wuwei	101	11	10.89	12	10.89	18	17.82	2	1.98

a chi-square 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

Antigens or antibodies to *D. immitis*, FeLV, and FIV were found in 3.04, 11.33, and 9.12 % of 362 cats, respectively; infection with both FIV and FeLV was the most common co-infection (29.82 %) (Table 2). Overall, 70 of 362 (19.34 %) examined cats were seropositive for *T. gondii* infection by MAT at the cutoff of 1:25 (Table 2). The antibody titers were diverse; the most frequent level was 1:200 (7.46 %), followed by 1:50 (3.04 %), 1:400 (2.76 %), 1:800 (2.49 %), 1:100 (1.93 %), and 1:25 (1.66 %).

Of 210 DNA samples, 24 were positive for the *T. gondii* B1 gene detected by PCR. Among the 24 positive DNA samples, 4 of them gave complete genotyping results, three were genotyped at 10 loci, one was genotyped at 9 loci (Table 3), and other samples which had less than 9 loci genotyped were not included. Genetic characterization of the 8 samples revealed two genotypes; 7 of the 8 samples were identified as ToxoDB#9, and the other sample was identified as ToxoDB#1 (type II). The genotyping results of these strains and 9 references are summarized in Table 3.

Only two factors were significantly associated with *T. gondii* infection. Stray cats (22.69 %) were more than 2 times (OR=2.428, 95 % CI 1.218–4.840, $P<0.001$) at risk of acquiring the infection compared to the pet cats (10.78 %). With regard to age, the likelihood of seroconversion increased with the age of the animal; cats above 18 months of age were nearly 3 times more likely than <6-month-old cats to have seroconverted (OR=2.939, 95 % CI 1.360–6.352, $P=0.005$). In the case of FIV, only age was associated with the likelihood of seroconversion to FIV. Cats above 18 months of age were over 6 times more likely than <6-month-old cats to have seroconverted (OR=6.912, 95 % CI 2.349–20.340, $P<0.001$). Moreover, cats above 18 months of age were more than 10 times (OR=10.680, 95 % CI 1.082–105.400, $P<0.001$) at risk of acquiring the *D. immitis* infection compared to the <6-month-old cats.

Discussion

In this investigation, the overall seroprevalence of *T. gondii* in the examined cats was 19.34 % (70/362), which was lower than that in cats in other places of China (Dubey et al. 2007; Qian et al. 2012) but similar to a previous study reporting an overall 21.3 % seroprevalence of *T. gondii* among cats in

Table 2 Prevalence of *Toxoplasma gondii*, feline immunodeficiency virus (FIV), feline leukemia virus (FeLV), and/or *Dirofilaria immitis* in cats in northwest China

Agent(s)	Number positive	Percentage positive (%)	Total positive for any listed agent	Percentage (%) co-infected out of cats with any listed agent
T. gondii	70	19.34	70	NA
D. immitis	11	3.04	11	NA
FeLV	41	11.33	41	NA
FIV	33	9.12	33	NA
FIV and FeLV	17	4.70	57	29.82
FIV and *D. immitis*	5	1.38	39	12.82
FeLV and *D. immitis*	4	1.10	48	8.33
FIV, FeLV, and *D. immitis*	4	1.10	61	6.56
T. gondii and FIV	17	4.70	86	19.77
T. gondii and FeLV	19	5.25	92	20.65
T. gondii and *D. immitis*	4	1.10	77	5.19
T. gondii, FIV, and FeLV	13	3.59	101	12.87
T. gondii, FeLV, and *D. immitis*	2	0.56	99	2.02
T. gondii, FIV, and *D. immitis*	1	0.28	89	1.12
T. gondii, FIV, FeLV, and *D. immitis*	0	0	126	0

NA not applicable

Lanzhou (Wu et al. 2011). However, the various methodologies used and greatly different sample sizes and sample populations in the countries surveyed may contribute to these difference so that it is difficult to compare reported seroprevalence.

Knowledge of genotyping *T. gondii* prevailing in feral and domestic felids is extremely valuable because humans can become infected through ingesting food and water contaminated with oocysts (Györke et al. 2011; Becker et al. 2012). Prior to the present study, no information on *T. gondii* genotypes from cats prevailing in northwest China is available. In this study, ToxoDB#9 was identified to be prevalent in this region. In previous studies, this same genotype was frequently identified in cats in many regions in China (Dubey et al. 2007; Chen et al. 2011; Qian et al. 2012; Wang et al. 2013; Tian et al. 2014), and it was also found in humans, pigs, goats, and other species (Zhou et al. 2011; Wang et al. 2012; Cong et al. 2015; Miao et al. 2015). Hence, ToxoDB#9 is considered to be a preponderant lineage prevalent in China.

FeLV and FIV infections in cats have been reported with various prevalences throughout the world (Vobis et al. 2003). The reported prevalence for FeLV and FIV in cats was 1.9–5.9 % in Canada (Little 2005), 3.2–3.6 % in Germany (Gleich et al. 2009), 3.5–10.4 % in England (Muirden 2002), 2.9–9.8 % in Japan (Maruyama et al. 2003), and 8.4–11.3 % in Italy (Bandecchi et al. 2006), respectively. Prevalence of retroviral infection shows obvious regional models in some countries. In the present study, FeLV was prevalent in these populations of cats with 11.33 % of the cats being antigen positive. Moreover, 9.12 % of the study cats were FIV positive. To our knowledge, this is the first report of retrovirus seroprevalence in cats in China. However, it is difficult to compare the present results with those of previous studies of FeLV and FIV infection in other regions due to differences in the study populations and types of cats, selection biases, diagnostic methods, and study design. Moreover, prevalence rates may change over time; thus, similar surveys should be repeated to monitor the trend.

Our results demonstrated that *D. immitis*, *T. gondii*, FIV, and FeLV seroprevalence was significantly associated with age, in agreement with other studies (Gleich et al. 2009; Wu et al. 2011; Sukhumavasi et al. 2012). The seroprevalence of these pathogens in adult cats was significantly higher than that in juvenile cats. This is probably because adult cats had more chance to contact with these pathogens comparing to juvenile cats, thus increasing the risk of infection.

The present study revealed the occurrence of *D. immitis*, FIV, and FeLV in cats in China for the first time. This study is also the first report of *T. gondii* genotypes (ToxoDB#9 and ToxoDB#1) in cats in northwest China. These findings indicated that further studies are warranted to investigate the epidemiology and detrimental impact of these pathogens in cats in other parts of China.

Table 3 Multilocus genotyping of *Toxoplasma gondii* isolates in cats in northwest China by PCR-RFLP analysis

Isolate ID	Host	Tissue	Location	SAG1	5′+3′SAG2	Alt.SAG2	SAG3	BTUB	GRA6	c22-8	c29-2	L358	PK1	Apico	Genotype
GT1	Goat		USA	I	I	I	I	I	I	I	I	I	I	I	Reference, type I, ToxoDB#10
PTG	Sheep		USA	II/III	II	II	II	II	II	II	II	II	II	II	Reference, type II, ToxoDB#1
CTG	Cat		USA	II/III	III	III	III	III	III	III	III	III	III	III	Reference, type III, ToxoDB#2
MAS	Human		France	u-1	I	II	III	III	III	u-1[a]	I	I	III	I	Reference, ToxoDB#17
TgCgCa1	Cougar		Canada	I	II	II	III	II	II	II	u-1[a]	I	u-2[a]	I	Reference, ToxoDB#66
TgCatBr5	Cat		Brazil	I	III	III	III	III	III	I	I	I	u-1[a]	I	Reference, ToxoDB#19
TgWtdSc40	W-t Deer		USA	u-1	II	II	II	II	II	II	II	I	II	I	Reference, ToxoDB#5
TgCatBr64	Cat		Brazil	I	I	u-1	III	III	III	u-1[a]	I	III	III	I	Reference, ToxoDB#111
TgToucan	Toucan		Costa Rica	u-1	I	II	III	I	III	u-2[a]	I	I	III	I	Reference, ToxoDB#52
TgCat1	Cat	Brain	Lanzhou	u-1	II	II	III	III	II	II	III	II	II	I	ToxoDB#9
TgCat2	Cat	Heart	Lanzhou	u-1	II	II	III	III	II	II	III	II	II	I	ToxoDB#9
TgCat3	Cat	Brain	Dingxi	u-1	II	II	III	III	II	II	III	II	II	I	ToxoDB#9
TgCat4	Cat	Brain	Dingxi	u-1	II	II	III	III	II	II	III	II	II	I	ToxoDB#9
TgCat5	Cat	Heart	Lanzhou	u-1	II	II	III	III	II	nd[b]	III	II	II	I	ToxoDB#9
TgCat6	Cat	Heart	Dingxi	nd[b]	II	II	III	III	II	II	nd[b]	II	II	I	ToxoDB#9
TgCat7	Cat	Heart	Wuwei	u-1	II	II	III	III	nd[b]	II	III	II	II	I	ToxoDB#9
TgCat8	Cat	Brain	Lanzhou	II/III	II	II	II	II	II	II	nd[b]	II	II	II	ToxoDB#1

[a] u-1 and u-2 represent unique RFLP genotypes, respectively

[b] nd means no data

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