Veterinary Parasitology 228 (2016) 13–16

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short communication

Comparison of genotypes of *Toxoplasma gondii* in domestic cats from Australia with latent infection or clinical toxoplasmosis

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ARTICLE INFO

Article history:
Received 18 November 2015
Received in revised form 29 May 2016
Accepted 4 June 2016

Keywords:
Feline
Genotype
Toxoplasma gondii
Toxoplasmosis
Australia

ABSTRACT

Whether *Toxoplasma gondii* genotype is associated with disease severity in naturally occurring toxoplasmosis in domestic cats is unknown. The aim of this study was to compare genotypes of *T. gondii* in latently infected cats with those in cats with clinical toxoplasmosis. Results of a PCR targeting the *B1* gene to detect *T. gondii* DNA were positive in tissue samples from 11 of 17 (65%) seropositive cats tested including four with clinical toxoplasmosis and seven with latent infections, as determined by serology, histologic findings and immunohistochemistry. Three of the four cats with clinical toxoplasmosis were immunosuppressed. Complete genotyping was performed in seven cats using PCR-RFLP at 12 loci (*SAG1*, *5′SAG2* and *3′SAG2*, *altSAG2*, *SAG3*, *BTUB*, *GRA6*, *c22-8*, *c29-2*, *L358*, *PK1* and *Apico*) and direct sequencing of the multi-copy *B1* gene. Partial genotyping using six loci was performed in one cat with latent infection. *T. gondii* type II (ToxoDB genotype #3) was determined in four cats with clinical toxoplasmosis and three cats with latent toxoplasmosis Novel *T. gondii B1* gene polymorphisms were detected in two strains (at nucleotide posititions 233, 366 and 595) and a *B1* gene polymorphism unique to Australia was identified in another (guanine/adenine at nucleotide position 378). One cat was co-infected with two or more type-II like strains at *3′SAG2*. The results of this study suggest that the infecting *T. gondii* genotype, based on these 12 loci, is not a determinant of clinical disease in cats naturally infected with *T. gondii* and type II strains are prevalent in Australia.

1. Introduction

Toxoplasma gondii infections in owned domestic cats are usually subclinical and severe toxoplasmosis is uncommon, as reflected by high seroprevalence among pet cats (Vollaire et al., 2005). Rarely, healthy cats with no detectable immune deficits or comorbidities develop severe clinical toxoplasmosis. Predisposing factors in these cases are not well understood.

The severity of clinical disease in some infected hosts is influenced by *T. gondii* genotype. Experimental infections of mice with clonal type I strains are fatal while those caused by Type II and III strains are less severe (Sibley and Boothroyd, 1992; Su et al., 2002). However, little is known about whether specific genotypes are over-represented in cats with naturally occurring clinical disease. Genotypes of *T. gondii* in healthy cats are regionally specific, such as a non-archetypal genotype, Chinese 1 (ToxoDB PCR-RFLP genotype #9), in China (Tian et al., 2014).

The aim of this study was to compare the genotypes of *T. gondii* in latently infected cats with those from cats with clinical toxoplasmosis. We applied multilocus PCR-RFLP genotyping based on 12 single locus markers and the multicopy *B1* gene.

2. Materials and methods

2.1. Samples

Tissue samples (skeletal muscle, forebrain, liver, and lung) collected prospectively at post-mortem in 10% neutral buffered formalin and also frozen at −80 °C from 16 cats seropositive for *T.gondii*-specific IgG (IDEXX NSW, Australia) and archived tissues from one cat previously diagnosed with disseminated toxoplasmosis were included for analysis. Sample collection was performed with owner consent and approval from the University of Sydney Animal Ethics Committee (N00/7-2013/3/6029).

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http://dx.doi.org/10.1016/j.vetpar.2016.06.008
0304-4017/

Histological examination of formalin fixed paraffin embedded tissues from all cats was performed after haemotoxylin and eosin (H&E) staining. Immunohistochemical (IHC) staining for *T. gondii* antigen was performed using *T. gondii* epitope-specific rabbit polyclonal antibody at 1:1000 dilution (RB-282-A; Thermo Fischer Scientific, Fremont, CA, US). Clinical toxoplasmosis was defined as histological detection of protozoal cysts and/or tachyzoites with positive IHC staining and associated inflammation. Latent infection was defined as absence of inflammation-associated protozoal cysts or zoites on histopathological and IHC examination of multiple tissues in seropositive cats.

2.2. Toxoplasma gondii B1 *genePCR amplification and multilocus genotyping*

DNA was extracted from three 25 mg aliquots of each tissue (muscle, lung, liver and brain) (DNeasy Blood and Tissue kit QIAGEN Germantown, MD). All DNA extractions included a negative control – molecular grade water (Life Technologies, Australia) (Donahoe et al., 2015). Presence of amplifiable DNA was confirmed by PCR of feline glyceraldehyde-3-phosphate dehydrogenase (Beatty et al., 2014). A diagnostic PCR amplifying the *B1* gene (530 bp) was used to detect *T. gondii* DNA (Parameswaran et al., 2010; Donahoe et al., 2014) in triplicate samples of each tissue type (Table 1).

T. gondii genotype, in cat tissues testing repeatedly positive on the *B1* PCR was then determined using a multilocus nested PCR to amplify *B1, SAG1, 5′SAG2, 3′SAG2, altSAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, alt.SAG2,* and *Apico* (Grigg and Boothroyd, 2001; Su et al., 2010) followed by DNA sequencing and virtual RFLP (Donahoe et al., 2014). PCR products were sequenced bi-directionally using amplification primers (Macrogen Inc., Seoul, South Korea). Chromatograms were visually verified using CLC Main Workbench 7.0.2 (QIAGEN, CLC bio, Aarhus, Denmark). Virtual RFLP was performed using NEB-Cutter (http://nc2.neb.com/) and compared to reference sequences of clonal *T. gondii* Types I (GT1; ToxoDB genotype #10), II (Me-49; ToxoDB genotype #1) and III (VEG; ToxoDB genotype #2) (Gajria et al., 2008). The reference sequence for the *B1* gene was AF179871.

Dinucleotide peaks were detected using the "secondary peak calling" function on CLC Main Workbench 7.0.2 with the cut-off of 20% of the maximum peak height (forward and reverse sequences) prior to assembly. Sequences were deposited in ToxoDB (EuroPathDB; http://toxodb.org/toxo/) and Genbank (KT881313–KT881388).

3. Results

3.1. *Cats with* T.gondii *infection*

T. gondii cysts and tachyzoites were detected in four of 17 cats (Supplementary Table 1). The *B1* gene PCR was positive in tissues from 11 of 17 cats tested (65%) including seven with latent infection and four with clinical toxoplasmosis (Table 1), with a varying degree of PCR reproducibility from examined DNA.

Eight cats (47%, 8/17; Cat 1–8) returned repeated satisfactory results in the *T. gondii B1* PCR assay (Table 1). Low concentratons of *T. gondii* DNA were suspected for Cats 9–11, based on PCR results (Table 1). Two of the four cats with clinical toxoplasmosis were diagnosed antemortem. In one of these, an FIV-infected cat, tachyzoites were detected in cytological preparations of liver from a fine-needle aspirate. Despite treatment with clindamycin (12.5 mg/kg IV q 12 h) and pyrimethamine (1 mg/kg PO q 12 h) the cat developed respiratory distress and was euthanized. In the other case, a cat receiving prednisolone (1 mg/kg PO q 24 h) and cyclosporin (6 mg/kgc PO q 24 h) for a severe chronic inflammatory polyarthropathy, tachyzoites and fungal hyphae were detected on cytology of pleural fluid (Supplementary Table 1).

In two cats clinical toxoplasmosis was diagnosed post-mortem, including a cat treated with multiagent chemotherapy for hepatic lymphoma and a cat with chronic progressive neurological signs (ataxia, circling and dysphagia) and multiple cranial nerve deficits.

3.2. Toxoplasma gondii *Type II in cats with clinical and latent infection*

Loci for *T.gondii* genotyping were amplified in eight of the 11 cats (73%) that tested positive on the *B1* gene PCR but not in the other three cats despite repeated attempts (Tables 2 and 3). Seven cats, including four with clinical toxoplasmosis were genotyped as ToxoDB genotype#3 using 12 loci. One cat, with latent infection could only be typed at six single copy loci (Table 2); the virtual RFLP suggested genotype ToxoDB#1, #3, #128 or #129. Comparison with reference *T. gondii* DNA sequences revealed unique polymorphisms (SNP, single nucleotide polymorphism) in the *3′SAG2* and *L358* locus of some strains (Table 3).

The *T. gondii* single copy *3′SAG2* locus from Cat 1 had dinucleotide peaks at nucleotide position 1222 and 1275 suggesting co-infection with more than one strain (Table 3). We then typed *T. gondii* from Cats 1–8 at the *B1* gene (a multicopy gene) locus because it has been shown to be variable between isolates (Costa et al., 2011). In five cats (Cats 1, 2, 5, 7, 8), *T. gondii* Type II/III alleles were present at the *B1* locus, while in three cats (Cats 3, 4, 6) *T. gondii* Type II/III-like alleles were detected (Table 3).

4. Discussion

This is the first study to characterise the genotypes of *T. gondii* in Australian cats where disease status was definitively identified. We did not find an association between genotype and clinical toxoplasmosis. While the findings in this study do not rule out the possibility that certain genotypes may be more virulent than others in cats, they suggest that factors that influence host susceptibility are more common determinants of clinical disease severity. Of the four cats with clinical toxoplasmosis, three were immunosuppressed.

Although experimental studies in cats have found associations between *T. gondii* genotype and disease severity, differences between studies in the inoculum dose, infecting parasite life stage (tachyzoites, oocysts or bradyzoites) and route of infection could be confounding variables (Parker et al., 1981; Powell and Lappin, 2001). Correlations between virulence and genotype are likely to be most applicable where the route of inoculation, life-stage of the parasite and inoculum dose mimics natural infection (Powell and Lappin, 2001).

Of eight other reported cases of clinical toxoplasmosis in cats where the infecting strain of *T. gondii* was genotyped, ToxoDB genotype #3 was detected in only one cat, from Switzerland, which, similar to cat 5 in this study, did not have concurrent disease (Spycher et al., 2011; Jokelainen et al., 2012; Dubey and Powell, 2013). Other genotypes detected including Type 12 clonal (ToxoDB genotype #4) in a cat from the United States and clonal Type II (ToxoDB genotype #1) in six cats from Europe (Jokelainen et al., 2012; Dubey and Powell, 2013) were all endemic to the region where cats were from and had also been detected in cats without clinical disease (Herrmann et al., 2010).

The high prevalence of ToxoDB genotype #3 in this study suggests it is endemic in Australia. This genotype is among the dominant genotypes found in Europe, Africa and North America and is the second most frequently identified genotype of all hosts worldwide after the clonal Type II genotype (ToxoDB#1) (Shwab et al., 2014). Few other Australian isolates of *T. gondii* have been