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ORIGINAL PAPER

Prevalence of *Toxoplasma gondii* infection in HIV-infected patients and food animals and direct genotyping of *T. gondii* isolates, Southern Ghana

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Abstract *Toxoplasma gondii* is of public health and veterinary importance causing severe diseases in immunocompromised individuals including HIV/AIDS patients and in congenital cases and animals. There is limited information on the epidemiology of *T. gondii* infection in humans, particularly HIV patients and food animals and the parasite genotypes in Ghana. A total of 394 HIV-infected patients from three hospitals were screened for *T. gondii* anti-IgG and IgM using ELISA. DNAs from blood samples of seropositve participants and 95 brain tissues of food animals were PCR assayed to detect *Toxoplasma gra6*. DNA positive samples were genotyped using multilocus nested polymerase chain reaction restriction fragment length polymorphism at 10 loci: *sag1*, *alt.sag2*, *sag3*, *btub*, *gra6*, *l358*, *c22-8*, *c29-2*, *pk1*, and *apico*. The overall seroprevalence was 74.37% (293/394). *Toxoplasma* DNAs were detected in 3.07% of the seropositive participants and 9.47% of the animals. Six of the human DNA positive samples were partly typed at *sag3*: 33.33, 50, and 16.67% isolates had type I, II, and III alleles, respectively. All nine isolates from food animals typed at nine loci except *apico* were atypical: six isolates were identical to ToxoDB #41 and #145, and one was identical to TgCkBrRj2 all identified in Brazil. The genotype of two isolates has not been reported previously and was named as TgCtGh1. *T. gondii* seroprevalence is high among the HIV-infected individuals with *T. gondii* circulating in Ghana being genetically diverse.

Keywords *Toxoplasma* · Human · Animals · HIV · Prevalence · Genotyping · Ghana

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Introduction

The protozoan *Toxoplasma gondii* (*T. gondii*) is a coccidian and an obligate intracellular parasite that infects warm-blooded animals (Howe and Sibley 1995). Felids, the only definitive host, are central in the transmission of this parasite. Infected felids excrete oocysts, the environmentally resistant form of *T. gondii* in their feces. In the environment, sporulated oocysts become infectious to many homeothermic vertebrates that act as the intermediate hosts. Within the intermediate hosts, *T. gondii* forms tissue cysts in many organs including those in the central nervous system, skeletal muscle, and visceral organs (Montoya and Liesenfeld 2004). Humans and animals become infected postnatally mainly by ingesting raw and undercooked infected meat containing viable *Toxoplasma* tissue cysts or food or drink contaminated with *Toxoplasma* oocysts excreted from the feces of infected cats. This makes toxoplasmosis a major foodborne (Torgerson et al.

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2015) and water-borne parasitic disease (Bahia-Oliveira et al. 2003; Bowie et al. 1997). In addition, humans may become infected through blood transfusion (Singh and Sehgal 2010) or organ transplantation (Montoya and Liesenfeld 2004). Pregnant females with active infection can pass the infection to the developing fetuses (Montoya and Liesenfeld 2004). Thus, *T. gondii* infection remains an important public health and veterinary medicinal significance.

It is estimated that about one third of the world human population is chronically infected, but only a small percentage of exposed immuno-competent individuals develop clinical manifestations during acute or chronic infection (Montoya and Liesenfeld 2004).The manifestations mainly include lymphadenopathy and neuropsychiatric symptoms including depression, schizophrenia, and suicide (Torrey et al. 2007; Wong et al. 2013). *T. gondii* mainly causes life-threatening diseases including encephalitis or death in immune-compromised individuals such as AIDS and cancer patients mostly due to reactivation of latent infection (Genot et al. 2007; Nissapatorn et al. 2004; Wang et al. 2015), also causing abortion or severe mortality in fetuses if transplacental infection occurs (Modrzejewska et al. 2016; Mohamed et al. 2014). In the animal husbandry industry, an increase in abortion, mortality, and associated medical cost due to toxoplasmosis has caused a considerable economic loss (Buxton et al. 2007; Cenci-Goga et al. 2013).

Recent studies have shown that apart from the host immune status, the genotype of the infecting parasite influences the course of the disease (Howe and Sibley 1995; Mercier et al. 2010). Therefore, studies on the genetic structure of *T. gondii* populations are very essential in providing information about the relationship between the genotypes and associated phenotypes, and such data will contribute to vaccine development for the management of this disease. Studies have revealed that *T. gondii* has diverse genetic populations comprising typical clonal (archetypical) and atypical clonal (non-archetypical) lineages (Ajzenberg et al. 2004; Dubey et al. 2008b) that have been grouped into 15 halogroups in six clades (Khan et al. 2007; Su et al. 2012). Pathogenicity analyses have revealed that different *Toxoplasma* strains exhibit different pathologies in humans and or virulence in mice due to polymorphism in proteins such as dense granule (GRA) and rhoptry (ROP) (Melo et al. 2011; Shwab et al. 2016). Among the existing genotypes, the three known distinct typical clonal types designated as I (ToxoDB genotype #10), II (clonal: #1 and variant: #3), and III (#2) have been isolated worldwide (Shwab et al. 2014). Type I lineage strains are highly virulent in mice whereas type II and III strains are intermediate or avirulent in mice. These clonal lineages are associated with asymptomatic and severe acute toxoplasmosis in humans and animals (Ajzenberg et al. 2002; Gebremedhin et al. 2014; Howe and Sibley 1995). The atypical strains include recombinants that contain alleles of the typical clonal types with or without unique alleles that are different from the alleles of the typical clonal types. Atypical isolates from both humans and animals also range from highly or less virulent to avirulent phenotype (Demar et al. 2007, 2012; Dubey et al. 2014).

The distribution of the genotypes varies with geographic regions. In Europe and North America, the *T. gondii* population is highly clonal predominated by typical clonal types II and III infecting both humans and domesticated animals (Shwab et al. 2014); also, a fourth clonal, type 12 (atypical), is predominant in wild animals from North America (Khan et al. 2011a; Yu et al. 2013). On the contrary, isolates from Central and South America are genetically diverse with no typical or atypical genotype dominating (Khan et al. 2011b; Shwab et al. 2014). Atypical #9 (Chinese 1) is predominant in Asia especially in China. On the other hand, types II and III predominate in Africa (Pappoe et al. 2016; Shwab et al. 2014). There are various methods of detecting the genotypes. The commonly used genotyping methods are multilocus polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) analysis (Su et al. 2006; Wang et al. 2015), microsatellite (MS) (Ajzenberg et al. 2009; Mercier et al. 2010), and multilocus sequence typing (MLST) (Vijaykumar et al. 2016). Both PCR-RFLP and MS are simple and cost effective compared with MLST. In this study, we chose PCR-RFLP against MS since it shows good sensitivity for detecting as few as 5 parasites in samples (Khan et al. 2005a) compared with at least 50 parasites per sample by MS (Ajzenberg et al. 2005).

Little has been known about the epidemiology of *T. gondii* and genotypes of this parasite in humans particularly HIV-infected patients and food animals in Ghana. The aim of the present study was to investigate the prevalence and genotypes of *T. gondii* from HIV-infected patients and cats, chickens, and goats which serve as the main food for many people in Ghana, and to verify whether the *T. gondii* genotypes circulating in domestic animals correspond to the genotypes detected in humans. Multilocus nested PCR-RFLP with 10 genetic markers was used to achieve a high resolution in identification. Samples were sequenced to resolve strain type whenever there were unclear enzyme digestion results.

Materials and methods

Study design and area

This was a case-case study conducted from May to August 2015. Human samples were collected from three hospitals in the Central region. The hospitals were Cape Coast Teaching Hospital (CCTH) which is the main referral hospital in the Central region and Cape Coast Metropolitan Hospital (CCMH) both located in Cape Coast Metropolis and Saltpond Municipal Hospital (SMH) located in the

Mfantseman District. Cape Coast is the capital city of the Central Region of Ghana with an estimated population of 169,894 (Ghana Statistical Service 2014a). It is located on latitude 5° 06′ N and longitudes 1° 15′ W. The estimated population of Mfantseman District is 196,563, and it is located within latitudes 5° 07′ to 5° 20′ N and between longitudes 0° 44′ to 1° 11′ W. The main occupations of these localities are fishing, farming, and trading (Ghana Statistical Service 2014a).

Sampling

A total of 394 HIV-infected inpatients and outpatients of all age groups who were attending services at CCTH, CCMH, and SMH in the Central region were consecutively recruited in this study. The study included antiretroviral therapy (ART) and co-trimoxazole-naive participants (newly confirmed HIV cases) and those who had been on ART for more than 3 months and on or off co-trimoxazole prophylaxis (old cases). HIV-infected individuals who had previous travel history outside Ghana were excluded. Data collected for all patients included demographical characteristics such as age and sex. The participants' WHO HIV clinical stage 1, 2, 3, or 4 information was also collected. The data were obtained from the patients or guardians through structured and standardized questionnaire interview, medical examination records, and informants including physician or medical personnel. Venous blood samples (2–5 ml) were drawn into sterile sodium citrate tubes and centrifuged at 2500×*g* for 10 min. The plasma was collected in Eppendorf tubes and stored at −80 °C until tested for *T. gondii* antibodies.

IgG and IgM antibody screening of patients for toxoplasmosis

The plasma samples of the patients were screened for IgG and IgM antibodies against *T. gondii* using commercially available enzyme-linked immunosorbent assay kits (ELISA) (Innovita Biological Technology, China) according to the manufacturer's instructions. Positive and negative controls supplied with the kits were included in each testing plate. Briefly, all the plasma was diluted 1:11 and incubated in *T. gondii* antigen-coated 96-well plates at 37 °C for 45 min. The plates were washed five times, 100 μl of horseradish peroxidase (HRP)-labeled conjugate was added to each well, and the plates were incubated at 37 °C for 45 min, washed five times, then substrates "A" and "B" 50 μl each were added and incubated at 37 °C for 15 min after which the reaction was terminated by adding 50 μl of stop solution (supplied with the kit). The optical density (OD) values were read at 450 nm using an automated microplate reader (Thermo Fisher Scientific, China). The critical value (cutoff) was determined from 0.10+ mean OD values of negative controls in each test as recommended by the manufacturer. A result equal to or greater than critical values was considered positive.

Animal samples

Animal samples were also collected from May to August, but it was a cross-sectional study. Free-range (FR) chicken and cat brains were purchased at Cape Coast from the Ministries Restaurant which serves as the eating place for many people and the FR chicken selling sector of Kotokruba Market which receives chicken from Cape Coast and beyond. Goat brains were purchased from the Kumasi Abattoir. The Abattoir which is government-approved receives livestock within and outside Kumasi and has qualified personnel. Kumasi is the capital city of Ashanti region and the second largest city of Ghana, with a population of 1,730,249. It is located between 6° 30′ and 7° 00′ N and 1° 30′ and 2° 00′ W at an altitude of 287 m above sea level (Ghana Statistical Service 2014b).

Fresh brain tissues of 40 cats and 30 goats slaughtered for human consumption were purchased on the day of killing. FR chicken ($n = 25$) were euthanized using sodium pentobarbital and commercial euthanasia solution. To avoid cross-contamination, each brain was placed in a sterile plastic bag, labeled, and stored at −20 °C until required for use.

DNA extraction and *Toxoplasma* GRA6 amplification by nested PCR

Genomic DNA was extracted from 200 μl of buffy coat of each of the blood samples of seropositive patients and 25 mg brain tissues from the domesticated animals using QIAamp DNA Blood and Tissue Mini Kits (Qiagen, Hilden, Germany). The manufacturer's instructions were followed. The concentration of the DNAs was determined using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Washington, USA). The DNA samples were screened for *T. gondii* using nested PCR targeting gra6 gene fragment. Nested PCR was chosen for this work due to its high specificity of DNA amplification. The PCR amplification was performed using a thermal cycler (TGradient 96, Biometra, Germany). Briefly, a total volume of 25 μl PCR mixture was prepared. It consisted of 12.5 μl 2× PCR Premix Taq (TaKaRa, Dalian, China), 1.0–2.0 μl (10 μM) each of the forward and reverse external primers gra6, and 1.5–3 μl of DNA sample (varied in concentration 22–280 ng). The reaction mixture was made up to 25 μl with deionized water. The first amplification steps involved initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 60 s, and extension at 72 °C for 90 s, and a final extension step at 72 °C for 10 min. The DNA products were then used as templates for the second PCR with internal primers using the same amplification settings except at the annealing stage where the temperature was adjusted to

60 °C. RH *T. gondii* DNA and nuclease-free water were used as positive and negative controls, respectively. The PCR products (5 μl each) were subjected to electrophoresis on a 1% agarose gel which was stained with 10 mg/ml ethidium bromide, and visualized with a UV transilluminator (Hema, Zhuhai, China).

Genetic characterization of *T. gondii* isolates by multilocus nested PCR-RFLP

DNA samples from both human and animals that gave positive GRA6 amplification were subjected to multilocus nested PCR-RFLP using these 10 genetic loci markers: *Sag1*, *Alt. Sag2*, *Sag3*, *Btub*, *Gra6*, *C22-8*, *C29-2*, *L358*, *PK1*, and *Apico* as previously described (Su et al. 2010). The PCR mixture preparation and nested PCR amplification conditions were the same as described above. This was followed by RFLP analysis in which the nested PCR-amplified products were digested using restriction enzymes (Thermo Scientific, Co., Ltd., China) specific for each genetic marker (Grigg et al. 2001; Khan et al. 2005b; Su et al. 2006). The manufacturer's instructions were followed. The digested products were visualized after electrophoresis with 3% agarose gels stained with 10 mg/ml ethidium bromide, and visualized with a UV transilluminator (Hema, Zhuhai, China). The following *T. gondii* strains were used as references: GT1, PTG, CTG, MAS, TgRsCr1, TgCatBr5, TgCatBr64 TgCgCa1, and TgCtWh3.

Data analysis

Statistical analysis was performed using the SPSS 19.0 software package (SPSS Inc., Chicago, Illinois). Chi-square was used to investigate associations among qualitative categorical variables. A P value less than 0.05 was considered statistically significant. The genotypes were compared with those listed in *Toxoplasma* data base (ToxoDB) at http://toxodb.org/toxo/.

Results

Age, sex, and clinical data associated prevalence of *T. gondii* infection in HIV-infected patients from three hospitals

The 394 HIV-infected patients enrolled in this study were made up of 24.37% males and 75.63% females, aged 3 to 76 (41.37 ± 12.19) years (Table 1). *T. gondii* antibodies were detected in 293 of the 394 HIV-infected patients with an overall seroprevalence of 74.37%. Of the 293 patients, 291 (99.32%) were seropositive for IgG only, 1 (0.34%) for IgM only, and 1 (0.34%) for both IgG and IgM. The distribution of seroprevalence for the three hospitals, CCTH, CCMH, and SMH, were 80.65, 78.57, and 64.43%, respectively. The difference in the positive rates in the three hospitals was statistically significant (χ^2 = 12.465, P = 0.002). The highest (80.28%) and the lowest (58.33%) seroprevalence was detected in the participants within the age groups 50–59 and ≤19, respectively. The seroprevalence was slightly higher in females (75.17%) than in males (71.88%). There was no significant difference in the seroprevalence of *T. gondii* in age groups (χ^2 = 4.691 P = 0.455) or gender (χ^2 = 0.413 P = 0.520) (Table 1). Table 2 shows the clinical data of the participants. The highest seroprevalence was observed in WHO stage 3 patients (86.21%) followed by stage 2 patients (75.64%), stage 1 patients (73.06%), and stage 4 patients (68.75%) with no statistically significant difference (χ^2 = 2.706, P = 0.439). No statistical significance was noted in the seropositive rates between new cases (76.60%) and old cases (74.06%) (χ^2 = 0.139, P = 0.709). Nested PCR amplification targeting the gra6 gene revealed the presence of *T. gondii* DNA in six (24.00%) WHO stage 3 patients and three (27.27%) stage 4 patients that were positive to *T. gondii* antibodies. The overall prevalence of *T. gondii* DNAs in the HIV/AIDS patients (stages 3 and 4) who had positive to anti-*T. gondii* antibodies was 25% (9/36). No *T. gondii* DNA was recovered from stage 1 and 2 patients. The overall prevalence of *T. gondii* DNAs in the participants that were seropositive to *T. gondii* infection was 3.07%. The difference was statistically significant (χ^2 = 38.759, P = 0.001).

Prevalence of *T. gondii* DNAs in the food animals

A total of 95 food animals (25 chicken, 40 cats, and 30 goats) were screened for *T. gondii* infection (Table 3). *T. gondii* DNAs were detected in six chickens and three cats, corresponding to a prevalence of 24.0 and 7.5%, respectively. No *T. gondii* DNAs were seen in all of the goats screened. The difference in the prevalence was statistically significant (χ^2 = 8.501, P = 0.008). The overall prevalence was 9.47%.

Multilocus nested PCR-RFLP genotyping of *T. gondii* isolates

The genotypes of the 18 *T. gondii* DNA samples including nine from animals and nine from humans were investigated (Table 4). Nine loci (except *Apico*) of each of the nine *T. gondii* isolates from animals were successfully amplified. Repeated attempts failed to amplify Apico. After enzyme digestion, it was observed that the *btub* locus of types II and III and *alt. sag2* locus of types I and II could not be distinguished. To resolve this, DNA sequencing was performed which indicated that two isolates, TgCtCC2 and 3, belonged to type II at *alt. sag2* and *btub* loci while six isolates (TgCtCC1 and TgCkCC1, 2, 4, 5, and 6) had type I allele at *alt. sag2*. Comparing our results with those listed at ToxoDB, we found

Table 1 Seroprevalence of *T. gondii* antibodies in HIV-infected individuals from Southern Ghana, West Africa

Data	CCTH (n = 217)	CCMH (n = 28)	SMH (n = 149)	Seroprevalence (%)	P value
Age group (years)					0.455
≤19	6	1	5	7/12 (58.33)	
20–29	21	8	15	33/44 (75.0)	
30–39	61	6	51	84/118 (71.19)	
40–49	76	8	41	96/125 (76.8)	
50–59	42	4	25	57/71 (80.28)	
≥60	11	1	12	16/24 (66.67)	
Gender					0.520
Male	49	4	43	69/96 (71.88)	
Female	168	24	106	224/298 (75.17)	
Total prevalence	175/217 (80.65)	22/28 (78.57)	96/149 (64.43)	293/394 (74.37)	

Seroprevalence in the hospitals (χ^2 = 12.465, P = 0.002)

that all the nine isolates from the food animals were atypical, displaying type I, II, and III (seven isolates) and type II and III (two isolates) allele combinations as shown in Table 4 and Fig. 1. Six isolates (TgCtCC1 and TgCkCC1, 2, 4, 5, and 6; 66.67%) had type I allele at *sag1*, *alt. sag2*, *btub*, *c22-8*, *c29-2*, *l358*, and *pk1*; type II allele at the *gra6* locus; and type III allele at *sag3*. Hence, these six isolates were identical to ToxoDB #41 and #145. The difference between #41 and #145 is that, at the *apico* locus, #41 has type I allele whereas #145 has type III allele. One isolate (TgCkCC3; 11.11%) was identical to TgCkBrRj2 displaying type I allele at *sag1*, *btub*, *c22-8*, *c29-2*, *l358*, and *pk1*; type II allele at *gra6*; and type III allele at *alt. sag2* and *sag3* loci; and two isolates (TgCtCC2 and 3; 22.22%) belonged to a new genotype which has not been previously reported. They had type II allele at seven loci (*alt. sag2*, *sag3*, *btub*, *gra6*, *c29-2*, *l358*, and *pk1*), type III allele at one locus (*c22-8*), and type II or III allele at one locus (*sag1*). We named this genotype TgCtGh1.

For the isolates from humans, only six (TgHuGh1, 2, 3, 4, 5, 6) of the nine isolates could be partly typed at the sag3 locus (Table 4): two isolates (TgHuGh1 and 2; 33.33%) were found to have type 1 allele; one isolate (TgHuGh3; 16.67%) had type III allele; and three isolates (TgHuGh4–6; 50%) had type II allele. DNA sequencing confirmed the RFLP results.

Discussion

We investigated the prevalence of *T. gondii* infection in HIV-infected patients and food animals and the genotypes of the infecting *T. gondii* isolates. The human seroprevalence varies in different countries and in different areas (Afonso et al. 2013; Zhou et al. 2011). The prevalence of majority of the studies from Latin American and African countries is above 50% and below 50%, respectively (Hammond-Aryee et al. 2014; Pappas et al. 2009). On the other hand, the US National Health and Nutrition Examination Survey (NHANES) studies have indicated a low seroprevalence of 12.5% (Jones et al. 2014). Also China has a low seroprevalence of 7.9% based on a nationwide survey (Zhou et al. 2011). In Ghana, seroprevalence ranges from 51.2 to 92.5% (Abu et al. 2015; Ayi et al. 2016b).

In HIV-infected patients, symptomatic life-threatening diseases mostly result from reactivation of latent infection due to reduction in CD4 cells below 200 cells/μl associated with HIV infection (Kodym et al. 2015; Nascimento et al. 2001; Nissapatorn et al. 2004) and a further reduction to 50 cells/μl has been reported to result in 35 times enhanced risk of reactivated toxoplasmosis (Laing et al. 1996). Therefore, early diagnosis of *T. gondii* infection is very necessary in all HIV-

Table 2 WHO clinical stage associated prevalence of *T. gondii* infection in HIV-infected individuals

WHO clinical stage	New case	Old case	Seroprevalence (%)	*T. gondii* DNAs prevalence (%)
Stage 1	10	261	198/271 (73.06)	0
Stage 2	23	55	59/78 (75.64)	0
Stage 3	10	19	25/29 (86.21)	6/25(24.00)
Stage 4	4	12	11/16 (68.75)	3/11 (27.27)
Total prevalence	36/47 (76.60)	257/347 (74.06)	293/394 (74.37)	9/293 (3.07)

Seroprevalence among the participants at different stages (χ^2 = 2.706, P = 0.439); seroprevalence between new case and old case participants (χ^2 = 0.139, P = 0.709); *T. gondii* DNA prevalence among the participants at different stages (χ^2 = 38.759, P = 0.001)

Table 3 Prevalence of *T. gondii* DNAs in food animals of Ghana

Regional area sampled	Host	No. tested	Prevalence (%)
Cape Coast	Chicken	25	6/25 (24.0)
Cape Coast	Cat	40	3/40 (7.5)
Kumasi	Goat	30	0
Total		95	9/95 (9.47)

(χ^2 = 8.501, P = 0.008)

infected individuals in order to prevent the complications of toxoplasmosis. However, little is known of *T. gondii* infection among HIV-infected victims in Ghana. In the present study, we detected antibodies against *T. gondii* in 293 out of 394 HIV-infected participants corresponding to a seroprevalence of 74.37% which is high compared with a similar report from Ghana, 57.6% (Ayi et al. 2016a). But a similarly high seroprevalence has been reported in HIV/AIDS-immunocompromised patients from Ethiopia and Iran in which 88.2 and 96.3% of positive *Toxoplasma* antibodies were reported, respectively (Rahimi et al. 2015; Yohanes et al. 2014). The criterion of seropositive was a positive test for either anti-*Toxoplasm*a IgG or IgM or both. We found that majority of the participants had chronic or past infection (99.32%; IgG only) whereas less than 1% had recent infection (0.34%, IgM only; 0.34%, IgM and IgG). Also, there was a significant difference in the seroprevalences in the three hospitals where the participants were recruited. This could be due to difference in sample size, eating habits, and pet keeping management and differences in livestock farming practices.

Chronic toxoplasmosis is treatable but not curable because the dormant bradyzoite stage in body tissues is impervious to host immunity and the available drugs (Khaw and Panosian 1995). Hence, the WHO has recommended co-trimoxazole as prophylaxis against opportunistic infection including toxoplasmosis among HIV-infected individuals (WHO 2005). We found no significant difference in the seroprevalence between new cases and old cases, and the majority of the participants in this study had chronic *T. gondii* infection, indicating that some of the old case participants acquired *T. gondii* infection before entry to the clinical care, during discontinuation of co-trimoxazole as a result of being stable on ART or poor prophylaxis adherence. Co-trimoxazole is reported to be effective in treating toxoplasmosis encephalitis in HIV/AIDS patients (Beraud et al. 2009; Torre et al. 1998), but its primary

Table 4 Genotyping of *T. gondii* isolates from food animals and HIV infected participants

Isolate ID	Host	Location	SAG 1	Alt. SAG 2	SAG 3	BTUB	GRA6	C22-8	C29-2	L358	PK1	APICO	Genotype
GT1	Goat	USA	I	I	I	I	I	I	I	I	I	I	Reference, ToxoDB #10 (type I)
PTG	Sheep	USA	II or III	II	II	II	II	II	II	II	II	II	Reference, #1 (type II)
CTG	Cat	USA	II or III	III	III	III	III	III	III	III	III	III	Reference, #2 (type III)
MAS	Human	France	u-1	II	III	III	III	u-1	I	I	III	I	Reference, #17
TgRsCr1	Toucan	Costa Rica	u-1	II	III	I	III	u-2	I	I	III	I	Reference, #52
TgCatBr5	Cat	Brazil	I	III	III	III	III	I	I	I	u-1	I	Reference, #19
TgCatBr64	Cat	Brazil	I	u-1	III	III	III	u-1	I	III	III	I	Reference, #111
TgCgCa1	Cougar	Canada	I	II	III	II	II	II	u-1	I	u-2	I	Reference, #66
TgCtWh3	Cat	China	u-1	II	III	III	II	II	III	II	II	I	Reference, Chinese 1, #9
Present study													
TgCkCC1, 2, 4–6	Chicken	Ghana	I	I	III	I	II	I	I	I	I	nd	Atypical; identical to #41 and #145
TgCtCC1,	Cat	Ghana	I	I	III	I	II	I	I	I	I	nd	Atypical; identical to #41 and #145
TgCtCC2, 3	Cat	Ghana	II or III	II	II	II	II	III	II	II	II	nd	Atypical; (new; TgCtGh1)
TgCkCC3	Chicken	Ghana	I	III	III	I	II	I	I	I	I	nd	Atypical; (TgCkBrRj2)
TgHuGh1,2	Human	Ghana	nd	nd	I	nd	nd	nd	nd	nd	nd	nd	Incomplete
TgHuGh3	Human	Ghana	nd	nd	III	nd	nd	nd	nd	nd	nd	nd	Incomplete
TgHuGh4–6	Human	Ghana	nd	nd	II	nd	nd	nd	nd	nd	nd	nd	Incomplete

nd no data because the quantity of the DNA was not sufficient

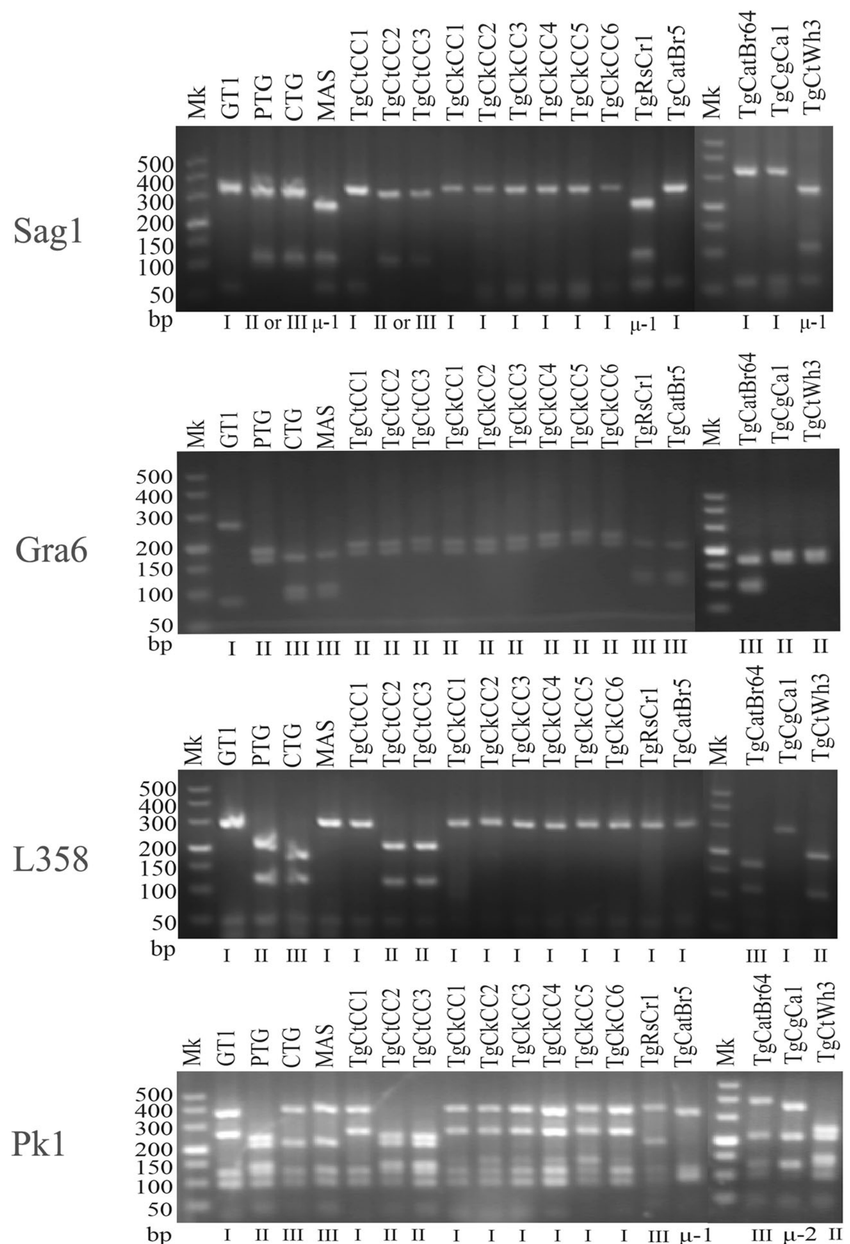

Fig. 1 Representative agarose gel image of PCR-RFLP genotyping (*Sag1*, *Gra6*, *L358*, and *PK1*). Samples IDs are at the *top*, and genotyping results are at the *bottom*. *Mk* is molecular marker. The reference strains are GT1, PTG, CTG, MAS, TgRsCr1, TgCatBr5, TgCatBr64, TgCgCa1, and TgCtWh3. The rest are isolates from food animals in this study

prophylaxis efficacy toward *T. gondii* infection is unknown. Therefore, further study is needed to evaluate the efficacy of co-trimoxazole as primary prophylaxis against *T. gondii* infection among HIV-infected individuals.

Since the primary aim of this study was genotyping, we detected *T. gondii* DNAs in 25% (9/36) of the HIV/AIDS-infected participants (stages 3 and 4) who showed positive *T. gondii* antibodies. The presence of *T. gondii* DNAs was closely associated with the HIV clinical status of the participants. Unfortunately, due to lack of resources at the hospitals, we were unable to perform CD4 counts for these participants. But based on the WHO clinical staging, it is most likely that these patients had considerably reduced CD4 cells. Our result is in agreement with other reports in which *T. gondii* DNAs were prevalent among HIV/AIDS patients (Alfonso et al. 2009; Ayi et al. 2016a; Nogui et al. 2009).

Food animals serve as reservoirs for human infection and are therefore of public health importance. *T. gondii* antibodies have been reported in chickens from Ghana (64%), Indonesia (24.4%), Italy (12.5%), Poland (30%), and Vietnam (24.2%) (Dubey et al. 2008a); 33.2% sheep and 26.8% goats from Ghana (Van der Puije et al. 2000); 32.1% pigs from Ethiopia (Gebremedhin et al. 2015); and 95.5% cats from Egypt (Al-Kappany et al. 2011). Moreover, the prevalence of *T. gondii* infection is found to be higher in food animals raised in non-confined rearing systems compared with their indoor-raised counterparts because outdoor-raised animals are more likely to ingest oocyst-contaminated feed and water (Dubey et al.

2012; Gebreyes et al. 2008; Xu et al. 2015). Hence, infection in food animals and other intermediate hosts requires the presence of cats, prey and predators of animals, or vertical transmission via placenta. This study is the first to investigate *T. gondii* infection in cats from Ghana. The result revealed 24.0 and 7.5% prevalence of *T. gondii* DNAs in chickens and cats, respectively. No *T. gondii* DNAs were detected in the goats which is in contrast with reports from Ethiopia (Gebremedhin et al. 2014) where *T. gondii* DNA prevalence of 45.45% was reported. A significant difference of positive rates of *T. gondii* DNAs was noted in the food animals with the overall prevalence of 9.47%.

From the *Toxoplasma* data base (ToxoDB), at least 231 *Toxoplasma* genotypes have been identified worldwide including the typical clonal types I, II, and III that were initially discovered in North America and Europe using six genetic markers (Howe and Sibley 1995). The identified genotypes were currently based on eight or more genetic markers, the majority typed at 10 genetic markers which have a high resolution in identification by distinguishing between typical and atypical lineages. In Ghana, there are only two genotyping studies. Dubey et al. (2008a) recovered two types (ToxoDB #132 and #137) from chickens using 10 genetic markers. In the other study, the isolates from HIV/AIDS subjects were typed based on three genetic markers which have very low identification resolution. The majority of the isolates were reported as clonal type II (93.8%, 76 isolates) while very few 1.2% (1 isolate) and 4.9% (4 isolates) were clonal type 1 and atypical, respectively (Ayi et al. 2016a). Additionally, an atypical genotype (I, III) was isolated from an HIV/AIDS Ghanaian man in France who had lived there for 5 years but it was not clear where he acquired the infection. The typing was based on five microsatellite loci (Genot et al. 2007).

In this study, we found that genotypes of all the nine isolates infecting the food animals were atypical. Six isolates, one from cat and five from chickens, were identical to ToxoDB #41 and #145 both identified in Brazil. ToxoDB #41 was recovered from domesticated chickens and capybara (*Hydrochaeris hydrochaeris*), a wild rodent mammal, and was found to be virulent in mice (Dubey et al. 2008c; Yai et al. 2009). ToxoDB #145 was recovered from another wild mammal, black-eared opossum rodent (*Didelphis aurita*), and was also found to be virulent in mice (Pena et al. 2011). One isolate from chicken was identical to TgCkBrRj2, also isolated from chicken in Brazil, reported from ToxoDB. Hence, the genotypic isolates from this study circulate in Brazilian *T. gondi*i population where the parasite population is highly diverse; these genotypes have widespread distribution. The novelty of this study is the identification of a new genotype which has not been previously reported in Ghana or elsewhere. It was identified in two cats and was named TgCtGh1 (TgCtCC2 and TgCtCC3). This new genotype shares the same allele pattern with #132 (TgCkGh2) at *sag1*, *btub*, *gra6*, and *pk1* loci but different pattern at *alt. sag2*, *sag3*, *c22-8*, *c29-2*, and *l358*. Also, it differs from #137 at *sag1*, *sag3*, *btub*, *c22-8*, *c29-2*, and *pk1* loci (Dubey et al. 2008a). Due to limited parasite DNAs, six of the isolates from the HIV-infected participants were partially typed at *sag3*. Based on which, all the six isolates were seen as typical clonal strains. Two strains (33.33%) had type I allele, three (50%) had type II allele, and one (16.67%) had type III allele. The genotypes of these isolates remain undefined due to the limited positive locus identified although DNA sequencing confirmed the RFLP results.

Conclusion

This study indicated high seroprevalence among HIV-infected individuals with high genetic diversity of *T. gondii* isolates from the food animals. The new genotype identified in this study suggests that there could be many more unique genotypes circulating in the Ghanaian environment. To better understand the population structure of *Toxoplasma* in Ghana, a much deeper sampling of a variety of hosts, including humans, from different geographic regions is necessary.

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Compliance with ethical standards The protocol of this study was approved by the Institutional Review Board of the Directorate of Research, Innovation and Consultancy (DRIC) of University of Cape Coast, Ghana, with reference number *UCCIRB/CHAS/2015/31*. Euthanasia of the chickens was in strict adherence with the Institutional Animal Care and use Committee (IACUC) guidelines, DRIC. Permission was obtained from the authorities of the various hospitals where human samples were collected. Participation in the study was voluntary without incentives. The purpose and procedures of the study were explained to all participants, and a written informed consent was obtained from each participating patient. Informed consent was obtained from parents or guardians for child participants. Additionally, permission was obtained from the Ghana Veterinary Service, Accra (Ref. No. VSD/RSS/01/15) for the use of the animals.

Competing interests The authors declared that they have no competing interests.

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