Isolation and characterization of *Toxoplasma gondii* from small ruminants (sheep and goats) in Chennai City, South India

Ajay Suryakant Satbige¹ · C. Sreekumar² · C. Rajendran³ · M. Vijaya Bharathi¹

Received: 7 November 2016 / Accepted: 21 March 2017 / Published online: 29 March 2017
© Indian Society for Parasitology 2017

Abstract The present study aimed for the isolation and genotyping of *Toxoplasma gondii* from small ruminants (sheep and goats). 14 out of 193 tissue samples (either brain and heart) tested positive by MDAT for anti-*T. gondii* antibodies, were selected and bioassayed, which resulted 4 samples positive for *T. gondii* after 40 days of post inoculation. Four samples consisting of 3 numbers of sheep and 1 number of goat tissues out of 14 samples detected by B1 PCR, were genotyped at SAG3 locus by nested polymerase chain reaction–restriction fragment length polymorphism technique (nPCR–RFLP). The results of the present study revealed that the four isolates designated as TgShIn19, TgShIn76, TgShIn77 and TgGtIn27 were circulating in small ruminants, were belonged to genotypes of type II (TgShIn19) and type III (TgShIn76, TgShIn77 and TgGtIn27) which are in concordance with the previously reported genotypes from other animal species and further this presumptive results indicating that the genotype II and III could be the predominant in different animal species including birds and humans in India.

Keywords *Toxoplasma gondii* · PCR–RFLP · Isolation · Genotyping · Small ruminants · South India

Introduction

Toxoplasma gondii is a single celled, intracellular protozoan parasite which infects all warm blooded animals including human (Dubey 2008). Felids are the definitive hosts, in which the parasites can undergo sexual replication to generate oocysts. A large numbers of unsporulated oocysts are shed in the cat's feces, however, oocyst shedding occurs for only 1–2 weeks. Within 1–5 days, oocysts in the environment sporulate and become infective. Infection with *T. gondii* is generally asymptomatic in immunocompetent individuals (Montoya and Liesenfeld 2004) but some individuals suffer from severe acquired forms of chorioretinitis (Burnett et al. 1998), or pulmonary forms of toxoplasmosis (Carme et al. 2002), which can be a life threatening when acquired congenitally (Wong and Remington 1994) or a consequence of reactivation in the immunocompromised patients (Luft and Remington 1992). Further, it can cause abortion and destructive inflammation that targets multiple organs including the brain and eyes (Wong and Remington 1994).

Sheep and goat rearing plays a major role in the economy of the farming community and fetal loss is a serious problem among them (Buxton and Rodger 2008; Dubey and Adams 1990; Mainar et al. 1996). Due to heavy numbers of population of stray cats in India, which may seed the environment with millions of oocysts of *T. gondii* (Dubey and Beattie 1988). According to 19th livestock census 2012, the total sheep populations are 65.06 million whereas the goat is 135.17 million all over India and most of them are being reared in free range or in semi-intensive system. So the possibility of ingestion of oocysts by these grazing ruminants are expected to be so high. The ingestion of undercooked infected lamb is considered to be one of the important source of infection to humans, which was

✉ M. Vijaya Bharathi

¹ Department of Preventive Medicine, Madras Veterinary College, Vepery, Chennai, Tamilnadu, India

² Post Graduate Research Institute in Animal Sciences, Tamil Nadu Veterinary and Animal Science University, Kattupakkam, Tamilnadu, India

³ Defence Food Research Laboratory, Defence Research and Development Organization (DRDO), Siddhartha Nagar, Mysore, Karnataka, India

identified as a high risk factor for *T. gondii* infection in pregnant women in Europe (Cook et al. 2000). Secondly, mutton and chevon are the very important sources of protein in India, thus acts as a potential mode of transmission to humans (Sacks et al. 1982) and recent findings revealed transmission through goat milk as another important risk factor (Dubey et al. 2011).

Early studies classified *T. gondii* into type I, II and III based on mouse virulence (Darde et al. 1992). A considerable number of studies from North America and Europe revealed three distinct lineages of type I, II and III in addition to recent type 12 (fourth clonal lineage) (Dubey et al. 2011; Khan et al. 2011). Limited studies from the African continent (Velmurugan et al. 2008; Al-Kappany et al. 2010), the Middle East and the Arabic peninsula, revealed mostly of type II and III (Salant et al. 2009; Dubey et al. 2010). In the Asian continent, various studies specially in China from animals (Yan et al. 2014; Tian et al. 2014; Li et al. 2015) and humans (Wang et al. 2013, 2015) revealed the predominance of Chinese I (ToxoDB#9) genotype with the limited numbers of archetypal lineages I, II and III (Tian et al. 2014; Wang et al. 2013, 2015; Zhou et al. 2009); in India, limited genotyping revealed types II and III (Sreekumar et al. 2003; Biradar et al. 2014), type I (Sudan et al. 2015). On the contrary, isolates from South America are highly diverse (Dubey et al. 2002, 2007; Lehmann et al. 2006; Rajendran et al. 2012; Shwab et al. 2014) and grouped them into different classification (Pena et al. 2008). Therefore, the present study has been focused on the isolation and genotyping of *T. gondii* circulating in small ruminants (sheep and goats) in and around Chennai metropolitan City.

Materials and methods

Samples and sampling area

14 number titre (136 numbers of sheep and 57 numbers of goats) of samples showed positivity by MDAT at 1:20 titre by MDAT out of 193 samples (either of lung, brain and heart) used for our previous study (Satbige et al. 2016) were utilized for the isolation and genotyping studies in *T. gondii* free BALB/c mice (Table 1).

Mouse bioassay studies

The tissue samples were prepared for the bioassay as per the method described (Dubey 1998). The experimental grouping was made into 14 groups with three animals per group that is free from *T. gondii* infection, Swiss albino mice, approximately 30 days old, weighing 20–25 g. 1 ml

of tissue digest was mixed with two broad spectrum antibiotic solutions (50 µg of streptomycin and 100 IU of benzyl penicillin per ml of fluid), were injected subcutaneously for further observation. All mice were not showed any visible symptoms of *T. gondii* infection but all subjected to MDAT after 40 dpi and the four groups of seropositive mice were examined for the presence of tissue cysts of *T. gondii* (Dubey et al. 1998). Ethical clearance was obtained from the institutional animal ethical committee (IAEC) to carry out the mouse bioassay experiment. The brain samples were collected in sterile vials and subjected to extraction of total genomic DNA using DNeasy Tissue Kit as per the manufacturer's instructions (Qiagen, Germany).

B1 PCR and SAG3 genotyping of *T. gondii* strains

The PCR targeting 194 bp of B1 gene was used for the detection of *T. gondii* (Angel et al. 1997) from the seropositive mice. Further, it was subjected to genotyping with SAG3 marker along with standard positive control such as GT1 (type I), PTG (type II), CTG (type III), TgCgCa1, MAS, TgCtBr5, TgCtBr64 and TgRsCr1 using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) according to the method described (Su et al. 2006).

Results

Fourteen positive samples (10 from sheep and 4 from goats, 14/193) at 1:100 titre were subjected to mouse bioassay for the isolation and genotyping studies (Table 1). Acute toxoplasmosis was not observed during this study. All mice were tested positive at 1:100 by MDAT at 40 dpi. There was no evidence of either cysts in the brain or tachyzoites in the lung, when the impression smear taken from all the mouse. The tissues collected from these four groups subjected to B1 PCR (194 bp) showed positivity. These were grouped into four isolates of *T. gondii* (TgShIn19, TgShIn76, TgShIn77 and TgGtIn27) based on SAG3 genotyping with PCR–RFLP which revealed one isolate of type II (TgShIn19) and three isolates of type III (TgShIn76, TgShIn77 and TgShIn27).

Discussion and conclusions

The sheep and goats so called “small ruminants” are mostly reared in free range rearing or semi intensive system in India. Therefore, the possibility of expected ingestion of oocysts of *T. gondii* from the environment could be high. A considerable number of seroprevalence studies in

Table 1 The results of the bioassay for the isolation of *T. gondii* from different tissue samples of small ruminants with seropositivity at 1:100 in MDAT

Sample no.	Sheep or goat tissues	Tissue to be bioassayed	Mouse inoculation after 35 dpi ^a	B1 PCR	Genotypes
S19	Sheep	Heart	No symptoms	Positive	TgShIn19
S76	Sheep	Heart	No symptoms	Positive	TgShIn76
S77	Sheep	Heart and brain	No symptoms	Positive	TgShIn77
G27	Goat	Heart	No symptoms	Positive	TgGtIn27

^a Days of post inoculation

India revealed 5–100% from various animal species (Devada et al. 1998; Selvaraj et al. 2007; Vijaya Bharathi et al. 2003, 2011; Hannah et al. 2013) and 8.8–37.3% in immunocompetent humans (Dhumne et al. 2007; Singh et al. 2014) in addition to a major outbreak of *T. gondii* in humans through water contamination (Manikandan et al. 2006). In the intensively managed sheep had lower antibodies titre than the semi-intensively managed sheep (Savio and Nieto 1995; Abu Samra et al. 2007; Romanelli et al. 2007; Ragozo et al. 2008) indicating that the seroprevalence is directly reflected on the management in the western countries. *T. gondii* is considered to be one of the important abortifacient in sheep and goats worldwide but little is known about this protozoa induced abortion in Indian livestock (Sharma et al. 2008). Some workers have reported presumptive *T. gondii* serological evidence of abortion in goats (Sharma and Gautam 1972; Srivastava et al. 1984).

In this study, higher numbers of sheep were infected with *T. gondii* as compared to goats, which may be associated with the presence of cats in the farm area, and the probability that a cat might shed oocysts on a sheep farm and farm premises (Weiland and Dalchow 1970; Dubey 1994). Excreted toxoplasma oocysts from the infected cats would remain infectious for a long time (Ghorbani et al. 1983). Since the information regarding genotypes circulating in small ruminants is obscure in India, this present study focussed mainly on the prevailing genotypes in sheep and goats. The four strains of *T. gondii* from 4 animals (3 no. of sheep and 1 no. of goat) out of 14 tissue samples (TgShIn19, TgShIn76, TgShIn77 and TgGtIn27) were mouse bioassayed. The resultant positive samples were genotyped on SAG3 marker revealed one strain belonged to type II (TgShIn19) and three strain belonged to type III (TgShIn76, TgShIn77 and TgGtIn27). Majority of the sheep harboured *T. gondii* as compared to goat in this limited study. The chosen genotyping marker, SAG3 is the most sensitive marker within 10 common genotyping set, thus it is the easiest to detect when parasite DNA concentration is limited (Khan et al. 2005). Our study is in agreement with the previously reported studies. Some of

the studies showed the genotypes of *T. gondii* circulating among backyard chicken species in India had revealed two type II and 5 type III genotypes based on SAG2 genotyping (Sreekumar et al. 2003), type I based on SAG3 (Sudan et al. 2015) and type III based on GRA6 (Biradar et al. 2014); Further, a recent study in the HIV (human immune deficiency virus) infected human patients, who succumbed to severe cerebral toxoplasmosis revealed recombinant genotypes of type II/III and type I/III (Vijaykumar et al. 2016). Further detailed studies are needed to accurately assess the genotypes circulating among small ruminants.

However, it is concluded that this study exhibited type II and III are the genotypes mainly circulating among small ruminants. Other studies from various other hosts such as chicken and human from India had revealed a similar findings. Therefore, it is presumptively concluded that the type II and III could be the predominant genotypes circulating among different hosts such as animals and birds including human.

Acknowledgements This study was funded by TANUVAS for master thesis work submitted to the Madras Veterinary College, Tamil Nadu, India. Authors would like to thank Dr. J. P. Dubey, Senior Scientist, USDA, USA and Dr. Sarman Singh, Professor, AIIMS, New Delhi for their help providing research materials and technical support.

References

- Abu Samra N, McCrindle CME, Penzhorn BL, Cenci-Goga B (2007) Seroprevalence of toxoplasmosis in sheep in South Africa. *J S Afr Vet Assoc* 78:116–120
- Al-Kappany YM, Rajendran C, Abu-Elwafa SA, Hilali M, Su C, Dubey JP (2010) Genetic diversity of *Toxoplasma gondii* isolates in Egyptian feral cats reveals new genotypes. *J Parasitol* 96:1112–1114
- Angel SO, Matrajt M, Margarit J, Nigro M, Illescas E, Pszeny V, Amendoeira MR, Guarnera E, Garberi JC (1997) Screening for active toxoplasmosis in patients by DNA hybridization with the ABGTg7 probe in blood samples. *J Clin Microbiol* 35:591–595
- Biradar SS, Saravanan BC, Tewari AK, Sreekumar C, Sankar M, Sudhakar NR (2014) Genetic characterization of *Toxoplasma gondii* isolates from chickens in India by GRA6 gene sequence analysis. *Acta Parasitol* 59:666–674

- Burnett AJ, Short SG, Isaac-Renton J, King A, Werker D, Bowie WR (1998) Multiple cases of acquired toxoplasmosis retinitis presenting in an outbreak. *Ophthalmology* 105:1032–1037
- Buxton D, Rodger SM (2008) Toxoplasmosis and neosporosis. In: Aitken ID (ed) *Diseases of sheep*, 4th edn. Wiley, Hoboken, pp 112–118
- Carne B, Bissuel F, Ajzenberg D, Bouyne R, Aznar C, Demar M, Bichat S, Louvel D, Bourbigot AM, Peneau C, Neron P, Darde ML (2002) Severe acquired toxoplasmosis in immunocompetent adult patients in French Guiana. *J Clin Microbiol* 40:4037–4044
- Cook AJ, Gilbert RE, Buffolano W, Zufferey J, Petersen E, Jenum PA, Foulon W, Semprini AE, Dunn DT (2000) Sources of *Toxoplasma* infection in pregnant women: European multi centre case–control study. European Research Network on Congenital Toxoplasmosis. *BMJ* 15:142–147
- Darde ML, Bouteille B, Pestre-Alexandre M (1992) Isoenzyme analysis of 35 *Toxoplasma gondii* isolates and the biological and epidemiological implications. *J Parasitol* 78:786–794
- Devada K, Anandan R, Dubey JP (1998) Serologic prevalence of *Toxoplasma gondii* in chicken in Madras. *Indian J Parasitol* 84:621–662
- Dhumne M, Sengupta C, Kadival G, Rathinaswamy A, Velumani A (2007) National seroprevalence of *Toxoplasma gondii* in India. *J Parasitol* 93:1520–1521
- Dubey JP (1994) Toxoplasmosis. *J Am Vet Med Assoc* 205:1593–1597
- Dubey JP (1998) Refinement of pepsin digestion method for isolation of *Toxoplasma gondii* from infected tissues. *Vet Parasitol* 74:75–77
- Dubey JP (2008) The history of *Toxoplasma gondii*—the first 100 years. *J Eukaryot Microbiol* 55:467–475
- Dubey JP, Adams DS (1990) Prevalence of *Toxoplasma gondii* antibodies in dairy goats from 1982 to 1984. *J Am Vet Med Assoc* 1990:295–296
- Dubey JP, Beattie CP (1988) *Toxoplasmosis of animals and man*. CRC Press, Boca Raton, pp 117–141
- Dubey JP, Lindsay DS, Speer CA (1998) Structures of *Toxoplasma gondii* tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. *Clin Microbiol Rev* 11:267–299
- Dubey JP, Graham DH, Blackston CR, Lehmann T, Gennari SM, Ragozo AM, Nishi SM, Shen SK, Kwok OC, Hill DE, Thulliez P (2002) Biological and genetic characterisation of *Toxoplasma gondii* isolates from chickens (*Gallus domesticus*) from Sao Paulo, Brazil: unexpected findings. *Int J Parasitol* 32:99–105
- Dubey JP, Sundar N, Gennari SM, Minervino AHH, Farias NAR, Ruas JL, Santos TRB, Cavalcante GT, Kwok OCH, Su C (2007) Biologic and genetic comparison of *Toxoplasma gondii* isolates in free-range chickens from the northern Pará state and the Southern state Rio Grande do Sul Brazil, revealed highly diverse and distinct parasite populations. *Vet Parasitol* 134:182–188
- Dubey JP, Pas A, Rajendran C, Kwok OC, Ferreira LR, Martins J, Hebel C, Hammer S, Su C (2010) Toxoplasmosis in sand cats (*Felis margarita*) and other animals in the breeding centre for endangered Arabian wildlife in the United Arab Emirates and Al Wabra wildlife preservation, the State of Qatar. *Vet Parasitol* 172:195–203
- Dubey JP, Velmurugan GV, Rajendran C, Yabsley MJ, Thomas NJ, Beckmen KB, Sinnett D, Ruid D, Hart J, Fair PA, Mc Fee WE, Shearn-Bochsler V, Kwok OC, Ferreira LR, Choudhary S, Faria EB, Zhou H, Felix TA, Su C (2011) Genetic characterisation of *Toxoplasma gondii* in wildlife from North America revealed widespread and high prevalence of the fourth clonal type. *Int J Parasitol* 41:1139–1147
- Ghorbani M, Hafizi A, Shegerfcar MT, Rezaian M, Nadim A, Anwar M, Afshar A (1983) Animal toxoplasmosis in Iran. *J Trop Med Hyg* 86:73–76
- Hannah V, Vijaya Bharathi M, Rajendran C, Shankar S, Senthil NR, Sathyabama K, Ganesan PI (2013) Detection of *Toxoplasma gondii* infection from the food animals by B1 gene based polymerase chain reaction. In: International conference proceedings cited VPVGFS, PLAFFS, TANUVAS, and Chennai, p 177
- Khan A, Su C, German M, Storch GA, Clifford DB, Sibley LD (2005) Genotyping of *Toxoplasma gondii* strains from immune compromised patients reveals high prevalence of type I strains. *J Clin Microbiol* 43:5881–5887
- Khan A, Dubey JP, Su C, Ajioka JW, Rosenthal BM, Sibley LD (2011) Genetic analyses of atypical *Toxoplasma gondii* strains reveal a fourth clonal lineage in North America. *Int J Parasitol* 41:645–655
- Lehmann T, Marcet PL, Graham DH, Dahl ER, Dubey JP (2006) Globalization and the population structure of *Toxoplasma gondii*. *Proc Natl Acad Sci USA* 103:11423–11428
- Li YN, Nie X, Peng QY, Mu XQ, Zhang M, Tian MY, Min SJ (2015) Seroprevalence and genotype of *Toxoplasma gondii* in pigs, dogs and cats from Guizhou Province Southwest China. *Parasites Vectors* 8:214
- Luft BJ, Remington JS (1992) Toxoplasmic encephalitis. *Clin Infect Dis* 15:211–222
- Mainar RC, De la Cruz C, Asensio A, Dominguez L, Vazquez-Boland JA (1996) Prevalence of agglutinating antibodies to *Toxoplasma gondii* in small ruminants of the Madrid region, Spain and identification of factors influencing seropositivity by multivariate analysis. *Vet Res Commun* 20:153–159
- Manikandan P, Madhavan B, Balasundaram MB, Andavar R, Venkatapathy N (2006) Outbreak of ocular toxoplasmosis in Coimbatore, India. *Indian J Ophthalmol* 54:129–131
- Montoya JG, Liesenfeld O (2004) Toxoplasmosis. *Lancet* 363:1965–1976
- Pena HFJ, Gennari SM, Dubey JP, Su C (2008) Population structure and mouse virulence of *Toxoplasma gondii* in Brazil. *Int J Parasitol* 38:561–569
- Ragozo AMA, Yai LEO, Oliveira LN, Dias RA, Dubey JP, Gennari SM (2008) Seroprevalence and isolation of *Toxoplasma gondii* from sheep from Sao Paulo State, Brazil. *J Parasitol* 94:1259–1263
- Rajendran C, Su C, Dubey JP (2012) Molecular genotyping of *Toxoplasma gondii* from Central and South America revealed high diversity within and between populations. *Infect Genet Evol* 12:359–368
- Romanelli PR, Freire RL, Vidotto O, Marana ER, Ogawa L, De Paula VS, Garcia JL, Navarro IT (2007) Prevalence of *Neospora caninum* and *Toxoplasma gondii* in sheep and dogs from Guarapuava farms, Parana State, Brazil. *Res Vet Sci* 82:202–207
- Sacks JJ, Roberto RR, Brooks NF (1982) *Toxoplasma* infection associated with raw goat's milk. *J Am Med Assoc* 248:1728–1732
- Salant H, Weingram T, Spira DT, Eizenberg T (2009) An outbreak of Toxoplasmosis amongst squirrel monkeys in an Israeli monkey colony. *Vet Parasitol* 159:24–29
- Satbige AS, Vijaya Bharathi M, Ganesan PI, Sreekumar C, Rajendran C (2016) Detection of *Toxoplasma gondii* in small ruminants in Chennai using PCR and modified direct agglutination test. *J Parasit Dis*. doi:10.1007/s12639-015-0713-x
- Savio E, Nieto A (1995) Ovine toxoplasmosis: seroconversion during pregnancy and lamb birth rate in Uruguayan sheep flocks. *Vet Parasitol* 60:241–247
- Selvaraj J, Murali Manohar B, Singh S, Balachandran C (2007) Seroprevalence of *Toxoplasma gondii* in buffaloes. *J Vet Parasitol* 21:41–42
- Sharma SP, Gautam OP (1972) Prevalence of *Toxoplasma* antibodies in sheep and goats in the area of Hissar, Haryana, India. *Trop Anim Health Prod* 4:245–248

- Sharma S, Sandhu KS, Bal MS, Kumar H, Verma S, Dubey JP (2008) Serological Survey of Antibodies to *Toxoplasma gondii* in Sheep, Cattle, and Buffaloes in Punjab, India. *J Parasitol* 94:1174–1175
- Shwab EK, Zhu XQ, Majumdar D, Pena HF, Gennari SM, Dubey JP (2014) Geographical patterns of *Toxoplasma gondii* genetic diversity revealed by multilocus PCR–RFLP genotyping. *Parasitology* 141:453–461
- Singh S, Munawwar A, Rao S, Mehta S, Hazarika NK (2014) Serologic prevalence of *Toxoplasma gondii* in Indian women of child bearing age and effects of social and environmental factors. *PLoS Negl Trop Dis* 8:e2737
- Sreekumar C, Graham DH, Dahl E, Lehmann T, Raman M, Bhalerao DP, Vianna MC, Dubey JP (2003) Genotyping of *Toxoplasma gondii* isolates from chickens from India. *Vet Parasitol* 118:187–194
- Srivastava AK, Singh B, Gupta SL (1984) Toxoplasmosis in sheep and goats and its correlation to postnatal mortality and abortion. *Livest Adv* 9:55–57
- Su C, Zhang X, Dubey JP (2006) Genotyping of *Toxoplasma gondii* by multilocus PCR–RFLP markers: a high resolution and simple method for identification of parasites. *Int J Parasitol* 36:841–848
- Sudan V, Tewari AK, Singh H (2015) Molecular characterization and sequence phylogenetic analysis of surface antigen 3 (SAG3) gene of local Indian isolates (Chennai and Izatnagar) of *Toxoplasma gondii*. *Rev Inst Med Trop Sao Paulo* 57:205–209
- Tian YM, Huang SY, Miao Q, Jiang HH, Yang JF, Su C, Zhu XQ, Zou FC (2014) Genetic characterization of *Toxoplasma gondii* from cats in Yunnan Province Southwestern China. *Parasites Vectors* 7:178
- Velmurugan GV, Dubey JP, Su C (2008) Genotyping studies of *Toxoplasma gondii* isolates from Africa revealed that the archetypal clonal lineages predominate as in North America and Europe. *Vet Parasitol* 155:314–318
- Vijaya Bharathi M, Mohamed Basheer A, Nedunchelliyan S, Singh S, Meenakchi Selvan MS (2003) Comparison of modified direct agglutination and indirect haemagglutination tests for detection of *Toxoplasma gondii* antibodies in goats in Chennai. *J Vet Parasitol* 17:49–51
- Vijaya Bharathi M, Kandavel E, Nedunchelliyan S, Muralimanohar B, Kumanan K (2011) Prevalence of *Toxoplasma* antibodies by using modified direct agglutination test in dogs in Chennai. *J Vet Parasitol* 25:162–164
- Vijaykumar BR, Lekshmi SU, Sai Kant R, Vaigundan D, Mahadevan A, Rajendran C, Shankar SK, Jayshree RS (2016) Genetic characterization of *Toxoplasma gondii* from autopsy proven cases of AIDS associated cerebral toxoplasmosis in South India. *Infect Genet Evol* 39:106–112
- Wang L, Chen H, Liu D, Huo X, Gao J, Song X, Xu X, Huang K, Liu W, Wang Y, Lu F, Lun ZR, Luo Q, Wang X, Shen J (2013) Genotypes and mouse virulence of *Toxoplasma gondii* isolates from animals and humans in China. *PLoS ONE* 8:e53483
- Wang L, He LY, Meng DD, Chen ZW, Wen H, Fang GS, Luo QL, Huang KQ, Shen JL (2015) Seroprevalence and genetic characterization of *Toxoplasma gondii* in cancer patients in Anhui Province Eastern China. *Parasites Vectors* 8:162
- Weiland VG, Dalchow W (1970) Toxoplasma infectionen bei haustieren in der turkey serologische untersuchungen im sabin-feldman test. *Berl Munch Tierarztl Wochenschr* 83:65–68
- Wong SY, Remington JS (1994) Toxoplasmosis in pregnancy. *Clin Infect Dis* 18:853–861
- Yan C, Liang LJ, Zhang BB, Lou ZL, Zhang HF, Shen X, Wu YQ, Wang ZM, Tang RX, Fu LL, Zheng KY (2014) Prevalence and genotyping of *Toxoplasma gondii* in naturally-infected synanthropic rats (*Rattus norvegicus*) and mice (*Mus musculus*) in eastern China. *Parasites Vectors* 7:591
- Zhou P, Zhang H, Lin RQ, Zhang DL, Song HQ, Su C, Zhu XQ (2009) Genetic characterization of *Toxoplasma gondii* isolates from China. *Parasitol Int* 58:193–195