INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan that parasitizes a variety of hosts, from birds to diverse mammals, including humans. The felidae are the final hosts and they are responsible for the shedding of oocysts through feces in the environment. Other animals play a role in the life cycle as intermediate hosts, and in these hosts the protozoa can stay in a latent form represented by tissue cysts. Urban rats are important for the epidemiology of toxoplasmosis acting as a source of infection to domestic cats (definite hosts) and to other carnivores and omnivores such as dogs and pigs.

Sangiorgi, in Italy, reported for the first time the presence of Toxoplasma gondii in the lungs of an albino laboratory rat. Eyles was the first to look for antibodies against T. gondii in wild rats captured in Tennessee, U.S.A. and obtained a seropositivity of 8%. Other researchers have studied the presence of T. gondii in rats using different techniques and have found a serological prevalence varying from 0.8% to 59%. Studies to isolate the parasite in rodents have found prevalences ranging from 0% to 12.5% in rats captured from different places.

T. gondii isolated from several animal species, although morphologically undistinguishable, differ with respect to virulence and pathogenicity. Such samples have been characterized based on the virulence established through morbidity and mortality in Swiss albino mice. Nowadays, molecular methods have been adopted to show the existence of genotypes within T. gondii species, designated as type I, II, III, who are most prevalent in North America and Europe. Recent data suggest a high genetic diversity of T. gondii strains in humans and animals from South America.

A study analyzing 164 isolates from different hosts of South America has grouped the isolates in 42 different genotypes. In Brazil, analysis of isolates from domestic animals has revealed four genotypes which were considered common clonal lineages in the country, called BrI, BrII, BrIII e BrIV.

The presence of infection by T. gondii in dog populations shows environmental contamination by oocysts eliminated by cats or contamination of food given to these animals. This highlights a possible risk of infection to the human population- as they share the same habitat.

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T. gondii genetic variation has high relevance to the understanding of transmission, immunogenicity and pathogenicity, thus the genetic diversity is important to better characterize the molecular epidemiology of toxoplasmosis35. Therefore, the aim of this research was to verify the presence of anti-T. gondii antibodies in urban sinantropic rodents and communicating dogs in areas of solid residual recycling in Londrina, Paraná State, and also characterize genotypically the T. gondii isolates from rodents.

MATERIAL AND METHODS

Place of study and sampling

Points for collection of animals and samples were areas of solid residual recycling and scrap yards in the five regions in the city of Londrina, Paraná State (North, South, East, West and Central). The number of rodents captured was calculated using the EP16 program36. For an infinite population, a prevalence of 50% was estimated, precision of 7.5% and significance level of 5%, resulting in 171 samples. The study was approved by the Ethical Committee in Animal Experimentation of the State University of Londrina (nº. 28/06).

Capture and rodents characterization

Rats were captured between May and December 2006, using cage mouse traps with a trigger activated by bait placed in its interior. Traps were assembled by the end of the afternoon in areas where there were traces of rodents passing by, such as: feces, fat stains, trails, or areas of great offer of food, and checked in the following morning. The species identification was performed based on external morphological characteristics such as the head format and length, tail, ear, posterior foot and body weight; in young animals, we consider that measures could be inferior to that of adults, but the identification was possible due the tail length, posterior foot and head format39. The rodents were classified, in the young adult age group, by the presence of well-developed sexual organs39.

Blood collection from dogs

Blood samples from dogs living in the areas where rodents were captured were collected by puncturing the cephalic or jugular vein using disposable syringes and 25x7 gauge needles. After the coagulum retraction, serum samples were stored in 1.5 mL polypropylene tubes and kept at -20 °C. In order to obtain epidemiological data, an epidemiological questionnaire was used, containing data concerning the rodent species, the area in which it was captured, type of trap and bait used, signs of presence of rodents, presence of communicating animals, and the association of these factors was analyzed.

Bioassay

Captured rats were submitted to anesthesia with ethyl ether for the blood collection from the brachial plexus and thereafter the animals were submitted to euthanasia. Serum samples were separated, stored and kept at -20 °C. In order to conduct the bioassay, fragments from the brain and liver were collected, ground and homogenized in graal, with the addition of sterile physiological solution containing antibiotic (1,000 UI of penicillin and 100 µg of streptomycin/mL). In order to perform the bioassay, the resulting solution was filtered in sterile gaze and 1 mL was inoculated intraperitoneally for every two Swiss albino female mice with an average weight of 25 g.

Mice were observed daily, and those presenting clinical symptoms were submitted to euthanasia for the collection of peritoneal liquid to check the presence of tachyzoites. In the absence of tachyzoites, the brain, liver and spleen were macerated and inoculated in two other mice. Six weeks after inoculation, asymptomatic animals were euthanatized, their blood was collected for serology and brain fragments were collected for the observation of tissue cysts. Mice were considered negative to T. gondii in the absence of parasites (tachyzoites or tissue cysts) and of specific serum antibodies37,40.

Serology

Serum samples from dogs, captured rats and bioassayed mice were submitted to the indirect fluorescence IgG anti-T. gondii antibodies test (IFAT), using tachyzoites of the RH strain as antigen41. Species-specific fluorescein isothiocyanate-labeled conjugates were used (Sigma Chemical Co. and Zimed) as well as positive and negative sera controls. Positive dilutions were ≥ 1:16 for both species37,40.

In order to obtain positive controls for the genus Rattus, three bi-monthly inoculations with live tachyzoites from the RH strain were performed intraperitoneally, in three albino R. norvegicus. The first inoculum contained 2 x 10³ tachyzoites/ mL, the second and third, 1 x 10³ tachyzoites/ mL. The rats did not present any clinical signs and were euthanatized 15 days after the third inoculation for the collection of blood. Serum samples used as negative controls were from albino R. norvegicus, prepared at the Central Vivarium of the State University of Londrina. After standardization, the titer of positive serum samples controls and conjugate were 1,024 and 300, respectively.

DNA extraction

DNA extraction was performed using tachyzoites obtained from peritoneal washes of mice according to Garcia et al.42. Briefly, the sample was homogenized, 300 µL were transferred to a microtube with the same volume of the extraction buffer (200 mM of NaCl, 20 mM of Tris, 50mM of EDTA, proteinase K 1 mg/mL, and 2% SDS) and incubated at 56 °C for 1h. After this, 300 µL of tamponed phenol were added and centrifuged at 13,000 x g during 5 minutes. The aqueous phase was transferred to another tube containing phenol: chloroform: isoamilic alcohol and centrifuged at 13,000 x g during 5 minutes. The DNA precipitation was performed using sodium acetate and ethanol43.

Polimerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP)

In order to determine the T. gondii genotypes, 12 markers SAG244, GRA645, CS341, SAG346, SAG1, SAG2-alt, C22-8, C29-2, L358, PK1, BTUB, Apico47 were used. The first amplification followed a previously described protocol and then a nested PCR was performed39. Eight T. gondii reference strains (GT1, PTG, CTG, TgCgCa1, MAS, TgCatBr5, TgCatBr64 and TgRsCr1) were used as controls. In case one or more markers were not amplified, isolates would be subjected to a 18S rDNA amplification, to exclude the possibility of other apicomplexan parasites closely related to T. gondii (Hammondia hammondi, Neospora caninum and Sarcocystis neurona)38.

Statistical analyses

For the tabulation of data obtained from the epidemiological questionnaires and from the bioassay and serology, the EPI6 software was used and the Chi-square test, the Fisher Exact test and the Odds
RESULTS

A total of 182 rodents were captured from 37 areas of storage and recycling of solid residuals. In 27 of these areas, there were communicating dogs, whose blood samples were also collected. There were also residences in 15 of these 37 places.

A total of 181 (99.4%) rodents captured belonged to the species Rattus rattus and one (0.6%) to the species Mus musculus, being 77 (42.3%) male and 105 (57.7%) female animals. One hundred and fifty rodents (82.42%) were classified as adults and 32 (17.58%) as young. Sixteen (8.8%) were positive for T. gondii, all R. rattus. In the bioassay, four strains were isolated, two from rats that yielded positive IFAT and two from IFAT-negative rats. Serological (IFAT) and bioassay results are presented in Table 1. The presence of anti-T. gondii antibodies was more frequent in male rats (p = 0.048; OR = 3.33; CI 95%;1.00 – 11.73). There was no significant difference regarding the region where the rats were captured within the city of Londrina (p = 0.106), nor with respect to the age group (p = 0.670). Among seven serological negative rats which were positive by bioassay, six were adults weighting between 150 and 250 g and one was young, weighting approximately 50 g.

Three of four samples from which the parasites were isolated (bioassay), mice presented clinical signs of toxoplasmosis approximately ten days post inoculation (d.p.i), followed by death. Few tachyzoites were recovered from the peritoneal cavity. Regarding the fourth sample, mice presented clinical signs of toxoplasmosis at 24 d.p.i. They were then euthanized and tachyzoites were recovered from the peritoneum.

Concerning the 61 partly-domiciled dogs examined, 50 (82.0%) did not have any defined breed and 27 (44.3%) were male. From the 43 dogs that had their ages informed, 14 (32.6%) were younger than one year old. The prevalence of positive IFAT in dogs was 70.5% (43/61) and one (0.6%) to the species R. rattus. Being 77 (42.3%) male and 105 (57.7%) female animals. One hundred and fifty rodents (82.42%) were classified as adults and 32 (17.58%) as young. Sixteen (8.8%) were positive for T. gondii, all R. rattus. In the bioassay, four strains were isolated, two from rats that yielded positive IFAT and two from IFAT-negative rats. Serological (IFAT) and bioassay results are presented in Table 1. The presence of anti-T. gondii antibodies was more frequent in male rats (p = 0.048; OR = 3.33; CI 95%;1.00 – 11.73). There was no significant difference regarding the region where the rats were captured within the city of Londrina (p = 0.106), nor with respect to the age group (p = 0.670). Among seven serological negative rats which were positive by bioassay, six were adults weighting between 150 and 250 g and one was young, weighting approximately 50 g.

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Table 1

| Rats Sample | IFAT Titer | Bioassay (Swiss albino mice) |
|-------------|-----------|----------------------------|
| 25          | -         | 16                         |
| 28          | 16        | -                          |
| 48          | 32.000    | -                          |
| 60          | -         | -                          |
| 61          | -         | +                          |
| 134         | 4.096     | -                          |
| 143         | 256       | +                          |
| 146         | 4.096     | +                          |
| 155         | 1.024     | -                          |
| 164         | 16        | -                          |
| 168         | 16        | -                          |
| 172         | 16        | -                          |
| 108         | -         | 16                         |
| 185         | -         | 64                         |
| 187         | -         | 16                         |
| 189         | -         | 16                         |

The analysis of the four T. gondii isolates with twelve genetic markers demonstrated a genetic diversity, classifying these isolates as atypical genotypes. The four isolates are genotypically identical to two strains (Table 1) originally isolated from cats in Paraná, Brazil. In all of the isolates, the amplification of the twelve markers was successful, so that the 18S rRNA amplification was not used for differentiation. The four rats in which T. gondii was isolated had weights between 150 and 200 g and had reproductive organs well defined, being classified as adults.

Table 2

Multilocus genotyping of Toxoplasma gondii strains isolated from urban rats, Londrina, PR, 2006

| Isolate | SAG1 | SAG2 | SAG3 | BTUB | GRA6 | c22-8 | c29-2 | L358 | PK1 | Apico | CS3 | # Toxo DB | PCR-RFLP Genotype | Genotypes References |
|---------|------|------|------|------|------|-------|-------|------|-----|-------|-----|-----------|---------------------|---------------------|
| 60      | I    | III  | III  | III  | III  | I     | I     | I    | III | III  | II  | #21       | TgCatBr10, 22, 23, 28, 31, 32, 37, TgCkBr95, TgCpBr29, TgRBr09 | 55, 49, 29, 47, 64 |
| 61      | I    | III  | III  | III  | III  | I     | I     | I    | III | III  | II  | #14       | TgCatBr15, TgCkBr82, 90, 153, TgCkCh1, TgCkCo2, TgCkVe3, TgCkCo14, TgCyW6, TgDgBr19, TgDgCo9, 12, 15, 18, | 65, 66, 49, 67, 68, 29, 69, 29, 32, 47 |
| 143     | I    | III  | III  | III  | III  | I     | I     | I    | III | III  | II  |           |                      |                     |
| 146     | I    | III  | III  | III  | III  | I     | I     | I    | III | III  | II  |           |                      |                     |
DISCUSSION

There was a predominance of the R. rattus species in the city of Londrina, which was already expected, since this species has shown a huge dispersion in the urban environment, due to the increase of high buildings and with false ceilings, technical galleries for passage of wires and cables allowing the existence of shelters. Despite the lack of statistical significance, male rats were more prevalent probably due to more exploratory habits related to the male gender. In natural areas, rodents are infected by T. gondii mainly by the ingestion of sporulated oocysts present in the environment or transplacental (congenital transmission). Rats are one of the most resistant animals to T. gondii, clinical manifestation is associated to immunosupressant factors, the type of strain, the amount of inoculum, the age of rats and the infecting form of T. gondii.

Ito et al. observed that rats fed with T. gondii oocysts from different isolates have seroconverted without presenting clinical signs, but later the presence of tissue cysts was demonstrated in several organs, mainly in the brain. Dubey et al. showed that adult rats inoculated with a few oocysts presented subclinical infection, but developed anti-T. gondii antibodies 29 d.p.i., with an increase in serological titers up to 75 d.p.i.

The prevalence of toxoplasmosis (8.8%) found in rodents in the city of Londrina is compatible with some studies conducted worldwide. Franti et al. in the State of California, USA, observed a seroprevalence of 4% in 160 urban rats, and of 38% in 47 cats. Mercier et al. described a prevalence of 1.96% in 766 domestics and peridomestic rodents in Niamey, Niger. In Brazil, some studies reported lower prevalences in urban sianintrópicos rodents; in Umuarama, Paraná State, none of 24 R. rattus and 19 Mus musculus showed positive results in the sorological tests, and in São Paulo, Muradian et al. established the prevalence of 0.46% in 217 captured rodents by means of bioassays. Higher prevalences were found in Costa Rica (30.4% Rattus), in Panama (23.3% R. norvegicus), in England (59% M. domesticus) and Pakistan (58.57% R. rattus; 36.66% M. musculus). In these studies, it is clear that both, theserological prevalence and the isolation of T. gondii in rats are lower than in other domestic species, and these results are compatible with those we found, as the prevalence in rats was 8.8% and in communicating dogs was 70.5%.

In this study, four rodents had T. gondii isolated, fact that evinced the importance of the bioassay to characterize the animals as a source of infection for predators such as dogs and cats. Dubey et al. studied the prevalence and genotyping of T. gondii in rats captured in Grenada, West Indies (Caribbean), from a total of 238 R. norvegicus, two (0.8%) were positive by the modified agglutination test (MAT ≥ 1:40) and there was only one isolation of T. gondii genotype III. Researchers have concluded that rats were not important to the epidemiology of toxoplasmosis in that island. Other studies conducted in Brazil also revealed low frequencies of T. gondii isolation in rats. Muradian et al. obtained one isolate from R. norvegicus captured in São Paulo. Araújo et al. obtained two isolates from R. rattus captured in Umuarama, Paraná, and afterwards they also obtained isolates from serum-negative animals as we did in our study. This could happen when the animal is infected early in the intrauterine life, through a vertical transmission. In this case the animals show non measurable antibody levels. Dubey showed that congenitally infected rats presented brain cysts after two months of age. During this time, some of the animals were serologically negative. Such a result has led to the conclusion that isolation of T. gondii is possible in rats with negative serology and that the proportion of infected rats with no detectable antibodies is unknown in the environment. This explains the fact that, in the present study, there were seven negative rats by serology that were positive in the bioassay. Of these, six rats were characterized as young adults, therefore the absence of antibodies could be attributed to a recent infection.

The prevalence of T. gondii in dogs that lived in the areas where the rats were captured is compatible with the one found in other studies conducted in the Paraná State ranging from 19.7% to 75.98% in dogs from urban areas and from 20.8% to 84.1% in dogs from rural areas. In this study we should also associate the difference in the prevalence of toxoplasmosis observed among rats and dogs, with the fact that the predominant rodent population in the city was of R. rattus; a species that presents arboreal habits and is only found in the ground when searching for food. The exposure to environments contaminated by oocysts is greater for dogs, that are frequently semi-domiciled and have a major dispersion area than rats; in addition they have a longer life span when compared to rodents. This exploratory habit of dogs also clarify the low prevalence in less than one year-old dogs comparing with older dogs, considering that older dogs have already had more chances of contact with T. gondii infectant forms in the environment.

Domestic cats are the main responsible for the environmental contamination and probably for the prevalence of toxoplasmosis in dogs in urban areas. The hunting of rats and birds is the main form of infection in cats, and the vital cycle induced after the ingestion of tissue cysts is the most efficient, since 97% of cats eliminate oocysts in a pre-patent period of three to ten days. In Brazil, the prevalence of infection by T. gondii in cats is high, varying from 18% to 84.4%.

Genotyping isolates of T. gondii by PCR-RFLP with multiple markers has a resolution similar to DNA sequencing, being simpler and presenting a lower cost. In this study, all markers were amplified in the four isolates, but they were not characterized as belonging to the three clonal lineages (I, II, III), usually found in North America and Europe. Nevertheless, the four genotypes are similar to those found in other studies carried in Brazil, with cats, chickens, goats, capybara, rats and dogs (Table 2). Among the four isolated strains, three showed virulence in mice, as they showed clinical signs of T. gondii infection ten d.p.i followed by death. These results are in accordance with the study carried by Pena et al. who showed that the genotype II in the CS3 marker is related to a higher virulence in mice. One of the isolates showed a low virulence, the mice presented signals of infection 24 d.p.i, despite the same genotype. Nevertheless, is well known that T. gondii virulence in mice does not depend only on the parasite lineage, but also to the infectious inoculum, the route of infection, and the parasite form.

The dominant rodent species in the solid residual storage and recycling area in the city of Londrina was R. rattus, which presents mainly arboreal habits. Prevalence of toxoplasmosis in captured rodents was lower than in dogs, however the prevalence found in dogs inhabiting the same area indicates the environmental contamination by oocysts, or the contamination of food given to these animals. In our study, the use of genotyping tools for the identification of isolates allowed the characterization of strains, that had been previously found in different
Brazillian animals. The parasite isolation from serological negative animals by the IFAT has demonstrated that the identification of antibodies in those animals is not sufficient to characterize these animals as sources of infection for cats.

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REFERENCES

1. Dubey JP. Toxoplasmosis of animals and humans. 2 ed. Maryland: CRC Press; 2010.
2. Dubey JP. Advances in the life cycle of Toxoplasma gondii. Int J Parasitol. 1998;28:1019–24.
3. Tenter AM, Heckerth AR, Weiss LM. Toxoplasma gondii: from animals to humans. Int J Parasitol. 2000;30:1217–58.
4. Vidotto O, Navarro IT, Mitsuka R, Freire RL. Estudos epidemiológicos da toxoplasmose em suínos da região de Londrina, PR. Semina Cien Agrárias. 1990;11:53–9.
5. Sangiorgi G. Toxoplasma ratti n. sp. Giorn Acad Med Torino. 1915;78:381–5.
6. Eyles DE. Toxoplasma in the Norway rat. J Parasitol. 1952;38:226–9.
7. Burridge MJ, Bigler WJ, Forrester DJ, Hennemann JM. Serologic survey for Toxoplasma gondii in wild animals in Florida. J Am Vet Med Assoc. 1979;175:964–7.
8. Childs JE, Seegar WS. Epidemiologic observations on infection with Toxoplasma gondii in three species of urban mammals from Baltimore, Maryland, USA. Int J Zoonoses. 1986;13:249–61.
9. Chinchilla M. Epidemiología de la toxoplasmosis en Costa Rica: importancia de los roedores domésticos. Rev Biol Trop. 1978;26:113–24.
10. Defeo ML, Dubey JP, Mather TN, Rhodes RC. Epidemiologic investigation of seroprevalence of antibodies to Toxoplasma gondii in cats and rodents. Am J Vet Res. 2002;63:1714–7.
11. Dubey JP. Comparative infectivity of oocysts and bradyzoites of Toxoplasma gondii for intermediate (mice) and definitive (cats) hosts. Vet Parasitol. 2006;140:69–75.
12. Franti CE, Riemann HP, Beihmner DE, Suther D, Howarth JA, Ruppanner R. Prevalence of Toxoplasma gondii antibodies in wild and domestic animals in northern California. J Am Vet Med Assoc. 1976;169:901–6.
13. Marshall PA, Hughes JM, Williams RH, Smith JE, Murphy RG, Hide G. Detection of high levels of congenital transmission of Toxoplasma gondii in natural urban populations of Mus domesticus. Parasitology. 2004;128(Pt 1):39–42.
14. Morsy TA, El Safty AH, Habib KS, Arafa MA, el Bahrawy AF, al Dakhil MM. Antibodies against Toxoplasma in commensal rodents trapped in Riyadh Region, Saudi Arabia. J Egypt Soc Parasitol. 1994;24:279–84.
15. Webster JP. Prevalence and transmission of Toxoplasma gondii in wild brown rats, Rattus norvegicus. Parasitology. 1994;108(Pt 4):407–11.
16. Dubey JP, Bhuiyat MI, Macpherson CNL, de Allie C, Chikweto A, Kwok OCH, et al. Prevalence of Toxoplasma gondii in rats (Rattus norvegicus) in Grenada, West Indies. J Parasitol. 2006;92:1107–8.
17. Dubey JP, Weigel RM, Siegel AM, Thulieze P, Kitron UD, Mitchell MA, et al. Sources and reservoirs of Toxoplasma gondii infection in 47 swine farms in Illinois. J Parasitol. 1995;81:723–9.
18. Frenkel JK, Hassanein KM, Hassanein RS, Brown E, Thulieze P, Quintero-Nunez R. Transmission of Toxoplasma gondii in Panama City, Panama: a five-year prospective cohort study of children, cats, rodents, birds, and soil. Am J Trop Med Hyg. 1995;53:458–68.
19. Mir NA, Chihabra MB, Bhardwaj RM, Gautam OP. Toxoplasma infection and some other protozoan parasites of the wild rat in India. Indian Vet J. 1982;59:60–3.
20. Ruiz A, Frenkel JK, Intermediate and transport hosts of Toxoplasma gondii in Costa Rica. Am J Trop Med Hyg. 1980;29:1161–6.
21. Dubey JP, Frenkel JK. Experimental toxoplasmosis in mice with strains producing oocysts. J Parasitol. 1973;59:505–12.
22. Mitsuka R, Navarro IT, Silva ACB, Breganò JW, Alifieri A, Jankevicius JV, et al. Toxoplasma gondii: II. Caracterização antigênica de taquizoítos de oito amostras. Braz J Vet Res Anim Sci. 1998;35:110–4.
23. Dardé ML. Biodiversity in Toxoplasma gondii. In: Gross U, editor. Parasitology. 1994;108(Pt 4):407–11.
24. Dardé ML, Bouteille B, Pestre-Alexandre M. Isoenzymic characterization of seven strains of Toxoplasma gondii by isoelectrofocusing in polyacrylamide gels. Am J Trop Med Hyg. 1988;39:551–8.
25. Howe DK, Sibley LD. Toxoplasma gondii comprises three clonal lineages: correlation of parasite genotype with human disease. J Infect Dis. 1995;172:1561–6.
26. Lehmann T, Blackston CR, Parmley SF, Remington JS, Dubey JP. Strain typing of Toxoplasma gondii: comparison of antigen-coding and housekeeping genes. J Parasitol. 2000;86:960–71.
27. Su C, Evans D, Cole RH, Kissinger JC, Ajikoka JW, Sibley LD. Recent expansion of Toxoplasma through enhanced oral transmission. Science. 2003;299:414–6.
28. Dubey JP, Sundar N, Gennari SM, Minervino AIH, Farias NA da R, Ruas JL, et al. Biologic and genetic comparison of Toxoplasma gondii isolates from 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. 2007;143:182–8.
29. Dubey JP, Velmurugan GV, Chockalingam A, Pena HFJ, de Oliveira LN, Leifer CA, et al. Genetic diversity of Toxoplasma gondii isolates from chickens in Brazil. Vet Parasitol. 2008;157:299–305.
30. Lehmann T, Marcet PL, Graham DH, Dahl ER, Dubey JP. Globalization and the population structure of Toxoplasma gondii. Proc Natl Acad Sci USA. 2006;103:11423–8.
31. Pena HFJ, Gennari SM, Dubey JP, Su C. Population structure and mouse-virulence of Toxoplasma gondii in Brazil. Int J Parasitol. 2008;38:561–9.
32. Rajendran C, Su C, Dubey JP. Molecular genotyping of Toxoplasma gondii from Central and South America revealed high diversity within and between populations. Infect Genet Evol. 2012;12:359–68.
33. Marshal JL, Navarro IT, Ogawa L, Oliveira RC. Soroepidemiologia da toxoplasmose em gatos e cães de propriedades rurais do município de Jaguapitã, estado do Paraná, Brasil. Cien Rural. 1999;29:99–104.
34. Navarro IT, Freire RL, Ogawa L, Kano FS, Vidotto O, Antibodies against Toxoplasma gondii in plasma of dogs seen at the UEL veterinary hospital. Epidemiol Santé Animale. 1997:2:47.
35. Navarro IT, Vidotto O, Tudury EA, Valiana CC, Freire RL. Prevalence of anticorpos anti-Toxoplasma gondii em cães atendidos no hospital veterinário da UEL-PR. Semina Cien Agrárias. 1992;13:66–9.
36. Romanelli PR, Freire RL, Vidotto O, Marana ERM, Oggawa L, De Paula VSO, et al. Prevalence of Neospora caninum and Toxoplasma gondii in sheep and dogs from Guarapuava farms, Paraná State, Brazil. Rev Vet Sci. 2007;82:202–7.
37. Sibley LD, Khan A, Ajoka JW, Rosenthal BM. Genetic diversity of Toxoplasma gondii in animals and humans. Philos Trans R Soc Lond B Biol Sci. 2009;364(1530):2749–61.
38. Dean AG, Dean JA, Coulombier D, Bredel KA, Smith DC, Burton AH, et al. Epi Info, Version 6: a word processing, database and statistics program for epidemiology on microcomputers. Atlanta: Centers for Disease Control and Prevention; 1994.
39. Brasil. Ministério da Saúde. Manual de controle de roedores. Brasília: Funasa; 2002.
40. Navarro IT, Vidotto O, Giraldi N, Freire RL. Toxoplasma gondii: isolamento a partir de carne e cérebro de suínos comercializados na região de Londrina, PR. Semina Cien Agrárias. 1992;13:15–8.
41. Camargo M. Introdução às técnicas de imunofluorescência. Rev Bras Patol Clin. 1973;10:143–71.
42. Garcia JL, Gennari SM, Machado RZ, Navarro IT. Toxoplasma gondii: detection by mouse bioassay, histopathology, and polymerase chain reaction in tissues from experimentally infected pigs. Exp Parasitol. 2006:113:267–71
43. Sambrook J. Molecular cloning: a laboratory manual. 2 ed. Nova York: CSHL Press; 1989.
44. Howe DK, Honoré S, Derouin F, Sibley LD. Determination of genotypes of Toxoplasma gondii strains isolated from patients with toxoplasmosis. J Clin Microbiol. 1997;35:1411–4.
45. Fazaeli A, Carter PE, Dardé ML, Pennington TH. Molecular typing of Toxoplasma gondii strains by GRA6 gene sequence analysis. Int J Parasitol. 2000;30:637–42.
46. Grigg ME, Granat J, Boothroyd JC, Margolis TP. Unusual abundance of atypical strains associated with human ocular toxoplasmosis. J Infect Dis. 2001;184:633–9.
47. Su C, Zhang X, Dubey JP. Genotyping of Toxoplasma gondii by multilocus PCR-RFLP markers: a high resolution and simple method for identification of parasites. Int J Parasitol. 2006;36:841–8.
48. Su, C, Shwab, EK, Zhou, P, Zhu, XQ, Dubey, JP. Moving towards an integrated approach to molecular detection and identification of Toxoplasma gondii. Parasitology, 2010;137:1-11.
49. Dubey JP, Navarro IT, Sreekumar C, Dahl E, Freire RL, Kawabata IH, et al. Toxoplasma gondii infections in cats from Paraná, Brazil: seroprevalence, tissue distribution, and biologic and genetic characterization of isolates. J Parasitol. 2004;90:721–6.
50. Dubey JP, Frenkel JK. Toxoplasmosis of rats: a review, with considerations of their value as an animal model and their possible role in epidemiology. Vet Parasitol. 1998;77:1–32.
51. Wallace GD. Intermediate and transport hosts in the natural history of Toxoplasma gondii. Am J Trop Med Hyg. 1973;22:436–64.
52. Ito S, Tsuoda K, Nishikawa H, Matsui T. Pathogenicity for several laboratory animals of Toxoplasma oocysts originated from naturally infected cats. Natl Inst Anim Health Q(Tokyo). 1975:15:122–7.
53. Dubey JP. Pathogenicity and infectivity of Toxoplasma gondii oocysts for rats. J Parasitol. 1996;82:951–6.
54. Mercier A, Garba M, Bonnabau H, Kane M, Rossi JP, Dardé ML, et al. Toxoplasmosis seroprevalence in urban rodents: a survey in Niamey, Niger. Mem Inst Oswaldo Cruz. 2013;108:399–407.
55. Araújo JB, da Silva AV, Rosa RC, MArttei RJ, da Silva RC, Richini-Pereira VB, et al. Isolation and multilocus genotyping of Toxoplasma gondii in seronegative rodents in Brazil. Vet Parasitol. 2010;174:328–31.
56. Muradian V, Ferreira LR, Lopes EG, Esermerini P de O, Pena HF de J, Soares RM, et al. A survey of Neospora caninum and Toxoplasma gondii infection in urban rodents from Brazil. J Parasitol. 2012;98:128–34.
57. Ahmad MS, Maqbool A, Mahmood-ul-Hassan M, Mushquat-ul-Hassan N, Anjum AA. Prevalence of Toxoplasma gondii antibodies in human beings and commensal rodents trapped from Lahore, Pakistan. J Anim Plant Sci. 2012;22:51–3.
58. Murphy RG, Williams RH, Hughes JM, Hide G, Ford NJ, Oldbury DJ. The urban house mouse (Mus domesticus) as a reservoir of infection for the human parasite Toxoplasma gondii: an unrecognised public health issue? Int J Environ Health Res. 2008;18:177–85.
59. Ferraroni JJ, Reed SG, Speer CA. Prevalence of Toxoplasma antibodies in humans and various animals in the Amazon. Proc Helminthol Soc Wash. 1980;47:148–50.
60. Lucas SRR, Hagisawa MK, Loureiro VS, Ikseki JYH, Birgel EH. Toxoplasma gondii infection in Brazilian domestic outpatient cats. Rev Inst Med Trop Sao Paulo. 1999;41:221–4.
61. Pena HFJ, Soares RM, Amaku M, Dubey JP, Gennari SM. Toxoplasma gondii infection in cats from São Paulo state, Brazil: seroprevalence, oocyst shedding, isolation in mice, and biologic and molecular characterization. Res Vet Sci. 2006;81:58–67.
62. Santos SM, Amaral V, Reboças MM, Drumond LS. Anticorpos anti-Toxoplasma detectados por hemaglutinação indireta em soros de gatos domésticos provenientes da capital do estado de São Paulo, Brasil. Biológico. 1983;49:163–5.
63. Silva JCR, Gennari SM, Ragozo AMA, Amazones VR, Magnabosco C, Vai LEO, et al. Prevalence of Toxoplasma gondii antibodies in sera of domestic cats from Guarulhos and São Paulo, Brazil. J Parasitol. 2002;88:419–20.
64. Yue LIO, Ragozo AMA, Soares RM, Pena HFJ, Su C, Gennari SM. Genetic diversity among capybara (Hydrochaeris hydrochaeris) isolates of Toxoplasma gondii from Brazil. Vet Parasitol. 2009;162:332–7.
65. Dubey JP, Cortés-Vecino JA, Vargas-Duarte JJ, Sundar N, Velmurugan GV, Bandini LM, et al. Prevalence of Toxoplasma gondii in dogs from Colombia, South America and genetic characterization of T. gondii isolates. Vet Parasitol. 2007;145:45–50.
66. Dubey JP, Gennari SM, Sundar N, Vianna MCB, Bandini LM, Yai LEO, et al. Diverse and atypical genotypes identified in Toxoplasma gondii from dogs in São Paulo, Brazil. J Parasitol. 2007;93:60–4.
67. Dubey JP, Patitucci AN, Su C, Sundar N, Kwok OCH, Shen SK. Characterization of Toxoplasma gondii isolates in free-range chickens from Chile, South America. Vet Parasitol. 2006;140:76–82.
68. Dubey JP, Su C, Cortés JA, Sundar N, Gomez-Marin JE, Polo LJ, et al. Prevalence of Toxoplasma gondii in cats from Colombia, South America and genetic characterization of T. gondii isolates. Vet Parasitol. 2006;141:42–7.
69. Dubey JP, Sundar N, Nolden CA, Samuel MD, Velmurugan GV, Bandini LA, et al. Characterization of Toxoplasma gondii from raccoons (Procyon lotor), coyotes (Canis latrans), and striped skunks (Mephitis mephitis) in Wisconsin identified several atypical genotypes. J Parasitol. 2007;93:1524–7.
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