IJP: Parasites and Wildlife 12 (2020) 42–45

Contents lists available at ScienceDirect

IJP: Parasites and Wildlife

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

Serological evidence of *Toxoplasma gondii* infection in *Melanosuchus niger* (Spix, 1825) and *Caiman crocodilus* (Linnaeus, 1758)

Flávia Batista Ferreira[a], Arlindo Gomes de Macêdo-Júnior[a], Carolina Salomão Lopes[a], Murilo Vieira Silva[a], Eliézer Lucas Pires Ramos[a], Álvaro Ferreira Júnior[a], Sérgio Netto Vitaliano[b], Fernanda Maria Santiago[a], André Luis Quagliatto Santos[c], José Roberto Mineo[a], Tiago Wilson Patriarca Mineo[a,*]

[a] *Laboratory of Immunoparasitology, Department of Immunology, Microbiology and Parasitology, Federal University of Uberlândia, Av. Pará, 1720, Uberlândia, CEP, 38400 902, Brazil*

[b] *Laboratory of Parasitic Diseases, Department of Veterinary Preventive Medicine and Animal Health, School of Veterinary Medicine and Zootechny, University of São Paulo, SP, 05508-270, Brazil*

[c] *Laboratory for Education and Research on Wild Animals (LAPAS), Faculty of Veterinary Medicine, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil*

ARTICLE INFO

Keywords:
Alligators
Toxoplasma gondii
Serology

ABSTRACT

Toxoplasma gondii is a protozoan with worldwide prevalence, known to affect a large variety of warm-blooded hosts. However, its ability to induce long-lasting infections in cold-blooded animals remains unclear. The most likely source of infection is through consumption of meat containing tissue cysts or by ingestion of food or water contaminated with oocysts. The current global climate change trend and the progressive degradation of natural habitats are prone to alter the distribution of ectotherm populations over a short period of time, which may favor contact between these animals and the protozoan. In association, alligator meat is considered a delicacy in many regions and its consumption has been previously related to a diversity of foodborne diseases. In that sense, we proposed in this study to search for specific antibodies against *T. gondii* in serum samples of two common species of alligators from the Brazilian fauna (*Melanosuchus niger* and *Caiman crocodilus*). We obtained the serum samples from 84 alligators from the Araguaia region, which were tested by agglutination assays that do not require species-specific secondary antibodies (Modified Agglutination Test – MAT; Indirect Hemagglutination Assay – IHA). From the 84 samples tested, eight (9.5%) were positive by MAT. From those, seven (87.5% of MAT+, 8.3% of the total) were also positive by IHA, reassuring a probable exposure of these animals to the parasite. Direct parasite detection in muscle fragments of one serologically reactive alligator did not yield positive results. Our results provide serological evidence that Brazilian alligators may be exposed to *T. gondii* and further studies should be performed to elucidate whether alligators are natural hosts of this ubiquitous protozoan parasite.

1. Introduction

Toxoplasma gondii is an intracellular protozoan parasite of the phylum Apicomplexa, distributed worldwide, known to infect an extensive range of warm-blooded animal species, including humans (Dubey and Jones, 2008; Elmore et al., 2010; Harker et al., 2015). Toxoplasmosis is asymptomatic in most cases, although severe clinical signs may occur in immunosuppressed individuals or congenitally infected offspring (Cenci-Goga et al., 2011). The infection typically occurs by ingestion of water/food contaminated with oocysts or consumption of meat from chronically infected hosts that bear tissue cysts (Jones and Dubey, 2012). Human and dog populations surveyed in the Brazilian Araguaia, a region of transition between the Amazon forest, savannah and marshland ecosystems, present a seroprevalence of over 60% to *T. gondii* (da Silva et al., 2015; Raimundo et al., 2015). There is no record to date on the seroprevalence rates of wildlife to *T. gondii* in the region. These studies are usually hampered – among other reasons – do to the difficulties in the obtention of species-specific reagents, relying mainly on assays that are based on primary antigen-antibody interactions, as agglutination tests (da Silva et al., 2014; Horta et al., 2018; Acosta et al., 2019).

Although mammals and birds are recognized as usual hosts of *T.*

* Corresponding author.
E-mail address: tiago.mineo@ufu.br (T.W.P. Mineo).

https://doi.org/10.1016/j.ijppaw.2020.04.008
Received 19 August 2019; Received in revised form 25 March 2020; Accepted 22 April 2020

gondii, it is still unclear whether cold-blooded animals would be susceptible to the infection (Stone and Manwell, 1969; Burridge et al., 1979; al Sadoon and el Bahrawy, 1998; Omata et al., 2005; Dubey et al., 2009; Harker et al., 2015). Warm-blooded animals maintain their body temperatures ranging from 35 to 42 °C, whereas cold-blooded animals – as reptiles – present body temperature variations according to their environment (Lance, 2003). It was demonstrated that Brazilian alligators presented body temperature variations between a minimum of 16.9 °C and a maximum of 37.9 °C during the year, with an average of 25.7 °C in cold seasons and 30.1 °C in warm seasons (Campos et al., 2005; Campos and Magnusson, 2011). For *T. gondii*, as expected by its host range, ideal temperature for replication is 37 °C (Omata et al., 2005). In classic experiments, while tachyzoites were able to replicate in Vero cells at 35 °C, the parasite was able to infect and survive inside insect cell lines at 29 °C – although proliferation was not observed in those conditions (Buckley, 1973).

Different studies have indicated that *T. gondii* infection can be transmitted to humans by consumption of meat products derived from several animal species, such as pigs, sheep, cattle, chicken, turkey and goats (Jones and Dubey, 2012). Reptile leather, meat and eggs are considered a delicacy appreciated by an increasing number of consumers, which has driven captive breeding and raising of species as alligators (Magnino et al., 2009; Hoffman and Cawthorn, 2012). The sustainable use and management of alligator species is already a reality in Brazil (Neto et al., 2007), especially in the Araguaia region, the fourth largest of South America in number of animals, which shelters alligator species as *Melanosuchus niger* and *Caiman crocodilus* (Aquino et al., 2009). Its contemporary economic prominence led to studies that show that the crocodilians may act as hosts of nematodes, trematodes, hemoparasites (*Hepatozoon sp* and *Trypanossoma sp*), amongst others (Junker et al., 2008; Tkach et al., 2008; Magnino et al., 2009; Waddle et al., 2009; La Grange et al., 2013; Marcili et al., 2013; Bouer et al., 2017). There are no reports to date that assess the risk factors of *T. gondii* infection in humans after consumption of reptile meat and eggs, but the finding of parasite DNA in tissues of other ectothermic animals may indicate a possible threat to human health (Dlugonska, 2017).

In this report, we aimed to detect specific antibodies against *T. gondii* in serum samples of alligators from the Araguaia region in Brazil.

2. Material and methods

2.1. Serum samples and ethical statement

A total of 84 serum samples from wildlife alligators of the species *Melanosuchus niger* and *Caiman crocodilus* were obtained in the Araguaia region, close to the city of Araguaiana, Mato Grosso state, Brazil (14°46′47.8″ S and 51°32′50.9″W), which presents stable warm temperatures throughout the year – with maximum average temperature of 27.7 °C (January) and minimum average of 24.9 °C (July). The animals were captured live, tagged, had their blood withdrawn and released back to nature. Blood was collected by puncture of the cervical vertebral sinus without causing any harm to the animals (Myburgh et al., 2014) and serum samples were stored at −20 °C in the Laboratory for Education and Research on Wild Animals (LAPAS), Federal University of Uberlândia (UFU), until the execution of the serological assays.

The Committee of Ethics in Animal Research of Federal University of Uberlândia approved the protocol (CEUA/UFU #117/10), which was conducted in accordance with the Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation. The protocol was also approved and licensed by the System of Authorization and Information about Brazilian Biodiversity (SISBIO, #24684-1).

2.2. Serological tests

2.2.1. Modified agglutination test (MAT)

The serum samples were primarily tested for anti-*T. gondii* antibodies using the modified agglutination test (MAT), as described previously (Seefeldt et al., 1989). Briefly, antigen preparation consisted of an adjusted suspension of *T. gondii* RH strain tachyzoites fixed with 6% formalin under agitation for 12 h at 4 °C. To perform the assay, the suspension with fixed tachyzoites was added (25 μl/well) in 96-well U-bottom microplates, following by the addition vol/vol of the alligator serum samples diluted 1:25 in PBS, along with mouse positive and negative controls. The plates were incubated at 37 °C in humid chamber for 12 h. Results were considered positive in the presence of notable agglutination of the parasites. The formation of a button with defined contour was considered as a negative result.

2.2.2. Indirect hemagglutination assay (IHA)

The indirect hemagglutination assay was performed using a commercial kit (Toxotest IHA, Wiener lab, Rosario, Argentina) according to the manufacturer's instructions. Briefly, the serum samples, along with positive and negative human controls supplied by the kit, were diluted from 1:8 to 1:64 and mixed with 25 μl of antigen-coated test erythrocytes in a 96-well U-bottom microtiter plates. The reaction was incubated at room temperature for 1h. Samples with agglutinated erythrocytes that covered more than 50% of the bottom of the wells were considered positive (Gyimesi et al., 2006; Luo et al., 2017).

2.3. Attempts to directly detect T. gondii in muscle samples from an alligator

We attempted direct detection of parasite DNA and isolation of the parasite in a single alligator with positive serology results that we had access to fragments of muscles from its tail, in accordance with protocols previously described (Dubey, 1998; Lopes et al., 2016). Briefly, dissociated tissue fragments were inoculated in mice by the intraperitoneal route, which were observed during 30 days for morbidity and survival. Blood samples were collected in the end of the experiment and evaluated for seroconversion by ELISA. Also, approximately 500 ?l of the processed tissue samples was added to a confluent monolayer of HeLa cells, to observe whether parasite multiplication would be detected during a 30-day period. A quantitative real time polymerase chain reaction (qPCR) using SYBR green detection system (Promega Co, Madison, WI, USA) was used for the attempts to detect parasite DNA in those tissue fragments, through the amplification of *T. gondii*'s Tg529 region (Forward: 5′-GCTCCTCCAGCCGTCTTG-3'; Reverse: 5′-CCTCAC CCTCGCCTTCAT-3′). The assays were performed in a real-time PCR thermal cycler (StepOnePlus, Thermo Scientific, EUA), along with a standard curve of known amounts of *T. gondii* DNA extracted from culture derived tachyzoites.

3. Results and discussion

T. gondii is present all over the world and serological assays have been widely used for the epidemiological characterization of the infection, through the definition of pre-exposure to parasitic antigens (Dard et al., 2016). In order to detect specific antibodies against *T. gondii* in alligators from the Araguaia Region, the 84 serum samples we obtained were assayed by the Modified Agglutination Test (MAT) – a reliable tool for the serological assessment in wild life species; and further confirmed by a commercial Indirect Hemagglutination Test (IHA), originally standardized for human serological diagnosis. Based on those techniques, we found that eight samples (8/84, 9.52%) tested positive by MAT as bearing specific antibodies to *T. gondii*, while the overall positivity found by IHA was 30.95% (26/84; Table 1). Seven samples (7/8, 87.5%) were concomitantly positive by MAT and IHA, indicating an increased likelihood of true serological positivity of those animals (Fig. 1). A description of the IHA titers found in MAT positive samples is presented in Table 2.

Analyzing the appropriateness of the serological assays adopted for this purpose, previous studies have used MAT or IHA to detect antibodies against *T. gondii* in wild animals, since agglutination techniques

Table 1
Summary of the serological results against Toxoplasma gondii in serum samples of Alligators from the Araguaia region, assayed by MAT and IHA.

Serological assay	Positive samples (n = 84)	%
MAT	8	9.52
IHA	26	30.95

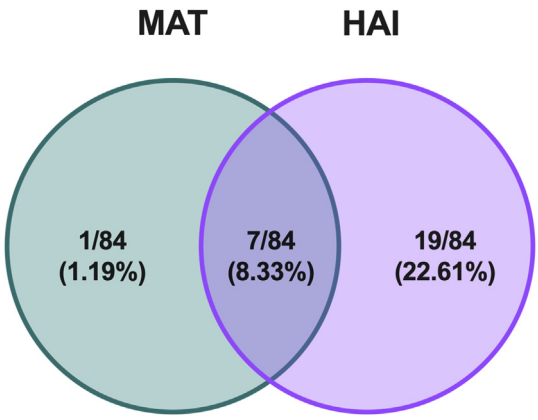

Fig. 1. Venn diagram displaying positivity of alligator serum samples to *Toxoplasma gondii* analyzed by serological methods MAT and IHA.

Table 2
Itemized IHA titers of alligator samples serologically positive to *Toxoplasma gondii* by MAT.

IHA titer	MAT positive samples (n = 8)	%
IHA-	1	12.5
IHA + (1:8)	2	25
IHA + (1:16)	1	12.5
IHA + (1:32)	1	12.5
IHA + (1:64)	3	37.5

do not require species-specific conjugates, and allows the detection of specific antibodies in practically any animal species (da Silva et al., 2014; Bolais et al., 2017). Comparative studies between distinct serological tests for *T. gondii* diagnosis have demonstrated discrepancies in the detection of specific antibodies, where the rates of seropositivity are related to the sensitivity and suitability of the employed tests (Silva et al., 1997; Elmore et al., 2014; Dard et al., 2016). It is our belief that the agreement between the tests in seven samples provides stronger evidence of previous contact of these animals with *T. gondii*.

One of the alligators that tested positive in the serological assays was in captivity, and had to be euthanized for unrelated reasons. We had access to a fragment of its tail musculature and attempted direct detection of the parasite by different techniques – qPCR, *in vitro* and *in vivo* isolation of live parasites. None of the attempts yielded positive results, as expected, due to the low likelihood imposed by the experimental setting – sampling of a single animal, access only to tail muscles, obtention of small fragments of tissue, among others. In addition, different research groups have reported evidences that the presence of this parasite in ectothermic animals may not be perennial as observed in warm-blooded species. It has been reported that experimental infections in reptiles and fish failed at their normal body temperatures, which fluctuates according to their environment (Frank, 1984; Omata et al., 2005). On the other hand, molecular analysis identified the presence of *T. gondii* DNA in the brain of five different species of snakes (Nasiri et al., 2016). Also, experimental infections in seven different reptile species with tachyzoites of the RH strain of *T. gondii* was shown to have limited success in animals kept between 18 and 26 °C, since the parasite was only observed in tissues or recovered by bioassay in mice after 7–14 days of infection – infections in alligators were not successful in this study (Stone and Manwell, 1969). On the other hand, horned toads (*Phrynosoma cornutum*) and red-eared turtles (*Pseudemys scripta elegans*) kept at 37 °C presented not only persistent infection, but also active replication of parasites until the end of the experimental period (~50 days) (Stone and Manwell, 1969).

Regarding the epidemiology of *T. gondii* and how alligators would fit in this context, the behavior of these animals intersects with the life cycle of the parasite. Crocodylians are predatory, and may prey upon crustaceans, small fish, other reptile, amphibians, and medium sized mammals and birds (Magnusson et al., 1987; Laverty and Dobson, 2013; Moldowan et al., 2016). Most likely, its carnivorous feeding habits will expose these animals – at least occasionally – to the ingestion of tissues bearing latent stages of the parasite. In addition, as shown in other conditions, the water they live in and its associated aquatic life may be contaminated with oocysts and serve as a source of infection (Dubey, 2004; Esmerini et al., 2010; Shapiro et al., 2015; Dlugonska, 2017).

Within the contemporary context of the global climate change currently underway, it is expected an expansion of the geographical spread and biodiversity of the crocodilian population, although concomitant destruction of their natural habitats may alter this trend (Mannion et al., 2015). Reptiles have a quick physiological adaptation to warming conditions, which is associated with increased genome-wide substitutions rates (Garcia-Porta et al., 2019). Within this perspective, considering the rise of temperatures worldwide, efficient adaptation of reptiles to warmer climates, the displacement of wildlife from their natural habitats and consequent augmented contact with humans and domestic animals, there is an increasing risk of augmented exposure and possible dissemination of *T. gondii* among these animals.

Taken together, we provide serological evidence that Brazilian alligators are exposed to *T. gondii* in their habitat, although further studies need to be performed to elucidate whether alligators are in fact natural intermediate hosts of *T. gondii*.

Acknowledgments

This work was supported by the Brazilian funding agencies Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brasil), Grant numbers: AUXPE Parasitologia 1348/2011, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brasil), Grant numbers: 307548/2016-3 and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, Brazil), Grant numbers: PPM 00547-17, APQ 01313-14.

References

Acosta, I.d.C.L., Souza-Filho, A., Muñoz-Leal, S., Soares, H., Heinemann, M.B., Moreno, L., González-Acuña, D., Gennari, S.M., 2019. Evaluation of antibodies against *Toxoplasma gondii* and *leptospira* spp. in magellanic penguins (*Spheniscus magellanicus*) on magdalena island, Chile. Vet. Parasitol.: Reg. Rep. Stud. 16, 100282.

al Sadoon, M.K., el Bahrawy, A.F., 1998. Blood parasites of five species of lizards trapped in Abha Province, Saudi Arabia. J. Egypt. Soc. Parasitol. 28, 899–905.

Aquino, S., Latrubesse, E., Bayer, M., 2009. Assessment of wash load transport in the Araguaia River (Aruana gauge station), Central Brazil. Lat. Am. J. Sedimentol. Basin Anal. 16, 119–128.

Bolais, P.F., Vignoles, P., Pereira, P.F., Keim, R., Aroussi, A., Ismail, K., Darde, M.L., Amendoeira, M.R., Mercier, A., 2017. *Toxoplasma gondii* survey in cats from two environments of the city of Rio de Janeiro, Brazil by Modified Agglutination Test on sera and filter-paper. Parasites Vectors 10, 88.

Bouer, A., Andre, M.R., Goncalves, L.R., Luzzi, M.C., Oliveira, J.P., Rodrigues, A.C., Varani, A.M., Miranda, V.F.O., Perles, L., Werther, K., Machado, R.Z., 2017. *Hepatozoon caimani* in *Caiman crocodilus yacare* (crocodylia, alligatoridae) from north pantanal, Brazil. Rev. Bras. Parasitol. Vet. 26, 352–358.

Buckley, S.M., 1973. Survival of *Toxoplasma gondii* in mosquito cell lines and establishment of continuous infection in Vero cell cultures. Exp. Parasitol. 33, 23–26.

Burridge, M.J., Bigler, W.J., Forrester, D.J., Hennemann, J.M., 1979. Serologic survey for *Toxoplasma gondii* in wild animals in Florida. J. Am. Vet. Med. Assoc. 175, 964–967.

Campos, Z., Coutinho, M., Magnusson, W.E., 2005. Field body temperatures of caimans in the Pantanal, Brazil. Herpetol. J. 15, 97–106.

Campos, Z., Magnusson, W.E., 2011. Emergence behaviour of yacare caimans (*Caiman*

crocodilus yacare) in the Brazilian Pantanal. Herpetol. J. 21, 91–94.
Cenci-Goga, B.T., Rossitto, P.V., Sechi, P., McCrindle, C.M., Cullor, J.S., 2011. Toxoplasma in animals, food, and humans: an old parasite of new concern. Foodb. Pathog. Dis. 8, 751–762.
da Silva, M.G., Vinaud, M.C., de Castro, A.M., 2015. Prevalence of toxoplasmosis in pregnant women and vertical transmission of *Toxoplasma gondii* in patients from basic units of health from Gurupi, Tocantins, Brazil, from 2012 to 2014. PloS One 10.
da Silva, R.C., Machado, G.P., Cruvinel, T.M., Cruvinel, C.A., Langoni, H., 2014. Detection of antibodies to *Toxoplasma gondii* in wild animals in Brazil. J. Venom. Anim. Toxins Incl. Trop. Dis. 20, 41.
Dard, C., Fricker-Hidalgo, H., Brenier-Pinchart, M.-P., Pelloux, H., 2016. Relevance of and new developments in serology for toxoplasmosis. Trends Parasitol. 32, 492–506.
Dlugonska, H., 2017. Are poikilothermic animals real hosts for *Toxoplasma gondii*? Ann Parasitol 63, 3–5.
Dubey, J., 1998. Refinement of pepsin digestion method for isolation of *Toxoplasma gondii* from infected tissues. Vet. Parasitol. 74, 75–77.
Dubey, J.P., 2004. Toxoplasmosis - a waterborne zoonosis. Vet. Parasitol. 126, 57–72.
Dubey, J.P., Jones, J.L., 2008. *Toxoplasma gondii* infection in humans and animals in the United States. Int. J. Parasitol. 38, 1257–1278.
Dubey, J.P., Mergl, J., Gehring, E., Sundar, N., Velmurugan, G.V., Kwok, O.C., Grigg, M.E., Su, C., Martineau, D., 2009. Toxoplasmosis in captive dolphins (*Tursiops truncatus*) and walrus (*Odobenus rosmarus*). J. Parasitol. 95, 82–85.
Elmore, S.A., Huyvaert, K.P., Bailey, L.L., Milhous, J., Alisauskas, R.T., Gajadhar, A.A., Jenkins, E.J., 2014. *Toxoplasma gondii* exposure in arctic-nesting geese: a multi-state occupancy framework and comparison of serological assays. Int J Parasitol Parasites Wildl 3, 147–153.
Elmore, S.A., Jones, J.L., Conrad, P.A., Patton, S., Lindsay, D.S., Dubey, J.P., 2010. *Toxoplasma gondii*: epidemiology, feline clinical aspects, and prevention. Trends Parasitol. 26, 190–196.
Esmerini, P.O., Gennari, S.M., Pena, H.F., 2010. Analysis of marine bivalve shellfish from the fish market in Santos city, Sao Paulo state, Brazil, for *Toxoplasma gondii*. Vet. Parasitol. 170, 8–13.
Frank, W., 1984. Non-hemoparasitic protozoans. In: Hoff, G.L., Frye, F.L., Jacobson, E.R. (Eds.), Diseases of Amphibians and Reptiles. Plenum Press, New York, pp. 259–384.
Garcia-Porta, J., Irisarri, I., Kirchner, M., Rodríguez, A., Kirchhof, S., Brown, J.L., MacLeod, A., Turner, A.P., Ahmadzadeh, F., Albaladejo, G., Crnobrnja-Isailovic, J., De la Riva, I., Fawzi, A., Galán, P., Göçmen, B., Harris, D.J., Jiménez-Robles, O., Joger, U., Jovanović Glavaš, O., Karış, M., Koziel, G., Künzel, S., Lyra, M., Miles, D., Nogales, M., Oğuz, M.A., Pafilis, P., Rancilhac, L., Rodríguez, N., Rodríguez Concepción, B., Sanchez, E., Salvi, D., Slimani, T., S'khifa, A., Qashqaei, A.T., Žagar, A., Lemmon, A., Moriarty Lemmon, E., Carretero, M.A., Carranza, S., Philippe, H., Sinervo, B., Müller, J., Vences, M., Wollenberg Valero, K.C., 2019. Environmental temperatures shape thermal physiology as well as diversification and genome-wide substitution rates in lizards. Nat. Commun. 10, 1–12.
Gyimesi, Z.S., Lappin, M.R., Dubey, J.P., 2006. Application of assays for the diagnosis of toxoplasmosis in a colony of woolly monkeys (*Lagothrix lagotricha*). J. Zoo Wildl. Med. 37, 276–280.
Harker, K., Ueno, N., Lodoen, M., 2015. *Toxoplasma gondii* dissemination: a parasite's journey through the infected host. Parasite Immunol. 37, 141–149.
Hoffman, L.C., Cawthorn, D.-M., 2012. What is the role and contribution of meat from wildlife in providing high quality protein for consumption? Animal frontiers 2, 40–53.
Horta, M.C., Guimaraes, M.F., Arraes-Santos, A.I., Araujo, A.C., Dubey, J.P., Labruna, M.B., Gennari, S.M., Pena, H.F.J., 2018. Detection of anti-*Toxoplasma gondii* antibodies in small wild mammals from preserved and non-preserved areas in the Caatinga biome, a semi-arid region of Northeast Brazil. Vet Parasitol Reg Stud Reports 14, 75–78.
Jones, J.L., Dubey, J.P., 2012. Foodborne toxoplasmosis. Clin. Infect. Dis. 55, 845–851.
Junker, K., Brooks, D.R., Boomker, J., 2008. *Proctocaecum gairhei* n. sp. (digenea: cryptogonimidae: acanthostominae) from *Gavialis gangeticus* (gmelin) in Nepal and a revised cladogram of proctocaecum baugh, 1957. Syst. Parasitol. 71, 229–236.
La Grange, L.J., Govender, D., Mukaratirwa, S., 2013. The occurrence of *Trichinella zimbabwensis* in naturally infected wild crocodiles (*Crocodylus niloticus*) from the Kruger National Park, South Africa. J. Helminthol. 87, 91–96.
Lance, V.A., 2003. Alligator physiology and life history: the importance of temperature. Exp. Gerontol. 38, 801–805.
Laverty, T.M., Dobson, A.P., 2013. Dietary overlap between black caimans and spectacled caimans in the Peruvian Amazon. Herpetologica 69, 91–101.
Lopes, C.S., Franco, P.S., Silva, N.M., Silva, D.A., Ferro, E.A., Pena, H.F., Soares, R.M., Gennari, S.M., Mineo, J.R., 2016. Phenotypic and genotypic characterization of two *Toxoplasma gondii* isolates in free-range chickens from Uberlandia, Brazil. Epidemiol. Infect. 144, 1865–1875.
Luo, H., Li, K., Shahzad, M., Zhang, H., Lan, Y., Xiong, X., 2017. Seroprevalence of *Toxoplasma gondii* infection in wild boars, wild rabbits, and wild chickens in hubei province, China. Kor. J. Parasitol. 55, 85–88.
Magnino, S., Colin, P., Dei-Cas, E., Madsen, M., McLauchlin, J., Nockler, K., Maradona, M.P., Tsigarida, E., Vanopdenbosch, E., Van Peteghem, C., 2009. Biological risks associated with consumption of reptile products. Int. J. Food Microbiol. 134, 163–175.
Magnusson, W.E., da Silva, E.V., Lima, A.P., 1987. Diets of amazonian crocodilians. J. Herpetol. 85–95.
Mannion, P.D., Benson, R.B., Carrano, M.T., Tennant, J.P., Judd, J., Butler, R.J., 2015. Climate constraints the evolutionary history and biodiversity of crocodylians. Nat. Commun. 6, 1–9.
Marcili, A., da Costa, A.P., Soares, H.S., Acosta, I.C., de Lima, J.T., Minervino, A.H., Gennari, S.M., 2013. First report of *trypanosoma* sp. in spectacled Caiman (*Caiman crocodilus*): morphological and phylogenetic relationships. ISRN Parasitol 2013, 328794.
Moldowan, P.D., Laverty, T.M., Emmans, C.J., Stanley, R.C., 2016. Diet, gastric parasitism, and injuries of Caimans (*Caiman, Melanosuchus*, and *paleosuchus*) in the Peruvian Amazon. S. Am. J. Herpetol. 11, 176–183.
Myburgh, J.G., Kirberger, R.M., Steyl, J.C., Soley, J.T., Booyse, D.G., Huchzermeyer, F.W., Lowers, R.H., Guillette Jr., L.J., 2014. The post-occipital spinal venous sinus of the Nile crocodile (*Crocodylus niloticus*): its anatomy and use for blood sample collection and intravenous infusions. J. S. Afr. Vet. Assoc. 85 01-10.
Nasiri, V., Teymurzadeh, S., Karimi, G., Nasiri, M., 2016. Molecular detection of *Toxoplasma gondii* in snakes. Exp. Parasitol. 169, 102–106.
Neto, J.V., Bressan, M.C., Rodrigues, E.C., Kloster, M.A., Aguil Santana, M.T., 2007. Physichist chymistry evaluation of swampland alligator meat (*Caiman yacare* Daudin 1802) of different ages. Cienc. E Agrotecnol 31, 1430–1434.
Omata, Y., Umeshita, Y., Murao, T., Kano, R., Kamiya, H., Kudo, A., Masukata, Y., Kobayashi, Y., Maeda, R., Saito, A., Murata, K., 2005. *Toxoplasma gondii* does not persist in goldfish (*Carassius auratus*). J. Parasitol. 91, 1496–1499.
Raimundo, J.M., Guimarães, A., Moraes, L.M.d.B., Santos, L.A., Nepomuceno, L.L., Barbosa, S.M., Pires, M.S., Santos, H.A., Massard, C.L., Machado, R.Z., 2015. *Toxoplasma gondii* and *Neospora caninum* in dogs from the state of Tocantins: serology and associated factors. Rev. Bras. Parasitol. Vet. 24, 475–481.
Seefeldt, S.L., Kirkbride, C.A., Dubey, J.P., 1989. Comparison of enzyme-linked immunosorbent assay, indirect fluorescent antibody test, and direct agglutination test for detecting *Toxoplasma gondii* antibodies in naturally aborted ovine fetuses. J. Vet. Diagn. Invest. 1, 124–127.
Shapiro, K., VanWormer, E., Aguilar, B., Conrad, P.A., 2015. Surveillance for *Toxoplasma gondii* in California mussels (*Mytilus californianus*) reveals transmission of atypical genotypes from land to sea. Environ. Microbiol. 17, 4177–4188.
Silva, D.A., Cabral, D.D., Bernardina, B.L., Souza, M.A., Mineo, J.R., 1997. Detection of *Toxoplasma gondii*-specific antibodies in dogs. A comparative study of immunoenzymatic, immunofluorescent and haemagglutination titers. Mem. Inst. Oswaldo Cruz 92, 785–789.
Stone, W.B., Manwell, R.D., 1969. Toxoplasmosis in cold-blooded hosts. J. Protozool. 16, 99–102.
Tkach, V.V., Strand, E.J., Froese, L., 2008. *Macroderoides texanus* n. sp. (Digenea: macroderoididae) from alligator gar, *Atractosteus spatula* in Texas. Parasitol. Res. 104, 27–33.
Waddle, A.R., Kinsella, J.M., Ross, J.P., Rojas-Flores, E., Percival, H.F., Forrester, D.J., 2009. Nematodes collected by gastric lavage from live *American alligators, Alligator mississippiensis*, in Florida. J. Parasitol. 95, 1237–1238.