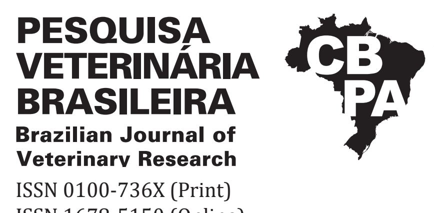

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Original Article
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Toxoplasma gondii, *Neospora caninum* and *Sarcocystis* spp. in species of naturally infected birds[1]

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ABSTRACT.- Alves M.E.M., Fernandes F.D., Bräunig P., Murer L., Minuzzi C.E., dos Santos H.F., Sangioni L.A. & Vogel F.S.F. 2022. ***Toxoplasma gondii*, *Neospora caninum* and *Sarcocystis* spp. in species of naturally infected birds.** *Pesquisa Veterinária Brasileira 42:e07026, 2022.* Laboratório de Doenças Parasitárias, Departamento de Medicina Veterinária Preventiva, Centro de Ciências Rurais, Universidade Federal de Santa Maria, Av. Roraima 1000, Prédio 63C, Bairro Camobi, Santa Maria, RS 97105-900, Brazil. E-mail: marta.elenamachado@gmail.com

Toxoplasma gondii, Neospora caninum and *Sarcocystis spp.* are parasites detected in tissues of domestic and wild animals. Birds are relevant in the life cycle and epidemiology of protozoa due to the wide variety of bird species, feeding and migratory habits. The aim of this study was the molecular detection of *T. gondii, N. caninum* and *Sarcocystis* spp. in several species of naturally infected birds. Therefore, samples of brain and heart tissue were collected from birds received and necropsied at the Central Laboratory for the Diagnosis of Avian Pathologies (LCDPA), undergoing DNA extraction and amplification by the polymerase chain reaction (PCR) of the 18S rRNA gene to *Sarcocystis* spp., NC5 gene for *N. caninum* and repetitive gene 529 base pairs for *T. gondii. N. caninum* was detected in two birds (02/65, 3.07%), in a brain sample of *Rupornis magnisrostris* (accession number: ON182081, 267pb) and in a brain and heart sample of *Dendrocygna bicolor* (accession number: ON211312, 267pb). DNA of the genus *Sarcocystis* was detected in three birds (03/65, 4.62%), and in the genetic sequencing *Sarcocystis* spp. (accession number: MW463929) in brain of *Nymphicus hollandicus* and *Sarcocystis speeri* (accession number: MW464125) in brain and heart of *Amazona aestiva.* Phylogenetic analysis revealed that *Sarcocystis* spp. formed a clade with *Sarcocystis* spp. that use skunk (*Didelphis aurita*) as definitive host and *Sarcocystis falcatula* that use Moluccan loris (*Trichoglossus moluccanus*) as intermediate host. *S. speeri* formed a clade with *S. speeri that used Mus musculus* as an experimental intermediate host and formed a clade with *Sarcocystis columbae, Sarcocystis corvusi, Sarcocystis halieti* and *Sarcocystis* sp. that affect bird species. *T. gondii* DNA was not detected in any tissue. This is the first report of DNA detection of *N. caninum, Sarcocystis* spp. and *S. speeri* in tissue samples for these bird species extending the list of intermediate hosts.

INDEX TERMS: *Toxoplasma gondii*, *Neospora caninum, Sarcocystis* spp., PCR, birds, brains, hearts, Apicomplexa.

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RESUMO.- [***Toxoplasma gondii*, *Neospora caninum* e *Sarcocystis* spp. em espécies de aves naturalmente infectadas.**] T*oxoplasma gondii*, *Neospora caninum* e *Sarcocystis* spp. são parasitas detectados em tecidos de animais domésticos e selvagens. As aves são relevantes no ciclo de vida e epidemiologia dos protozoários devido à grande variedade de espécies de aves, hábitos alimentares e migratórios. O objetivo deste estudo foi a detecção molecular de *T. gondii*, *N. caninum* e *Sarcocystis* spp. em diversas espécies de aves naturalmente infectadas. Portanto, amostras de tecido de cérebro e coração foram

coletados de aves recebidas e necropsiadas no Laboratório Central de Diagnóstico de Patologias Aviárias (LCDPA), sendo submetidas a extração de DNA e amplificação pela reação em cadeia da polimerase (PCR) do gene 18S rRNA para *Sarcocystis* spp., gene NC5 para *N. caninum* e gene repetitivo 529 pares de bases para *T. gondii*. *N. caninum* foi detectado em duas aves (02/65; 3,07%), em amostra de cérebro de *Rupornis magnisrostris* (número acesso: ON182081, 267pb) e em amostras de cérebro e coração de *Dendrocygna bicolor* (número acesso: ON211312, 267pb). DNA do genero *Sarcocystis* spp. foi detectado em três aves (03/65; 4,62%), sendo que no sequenciamento genético foram identificados *Sarcocystis* spp. (número acesso: MW463929) em cérebro de *Nymphicus hollandicus* e *Sarcocystis speeri* (número acesso: MW464125) em cérebro e coração de *Amazona aestiva.* A análise filogenética revelou que *Sarcocystis* spp. formou um clado com *Sarcocystis* spp. que utilizam gambá (*Didelphis aurita*) como hospedeiro definitivo e *S. falcatula* que utilizam Lóris-molucano (*Trichoglossus moluccanus*) como hospedeiro intermediário. *S. speeri* formou um clado com *S. speeri* que utilizou *Mus musculus* como hospedeiro intermediário experimental e formou um clado com *Sarcocystis columbae*, *Sarcocystis corvusi*, *Sarcocystis halieti* e *Sarcocystis* sp. que afetam espécies de aves. O DNA de *T. gondii* não foi detectado em nenhum tecido. Este é o primeiro relato de detecção de DNA de *N. caninum*, *Sarcocystis* spp. e *S. speeri* em amostras de tecido para essas espécies de aves estendendo a lista de hospedeiros intermediários.

TERMOS DE INDEXAÇÃO: *Toxoplasma gondii*, *Neospora caninum, Sarcocystis* spp., PCR, aves, cérebro, coração, Apicomplexa.

INTRODUCTION

Toxoplasma gondii, *Neospora caninum* and *Sarcocystis* spp. are widespread protozoa Apicomplexa organisms with heteroxenous life cycles with capacity of infecting a variety of domestic and wild animals and different degree of clinical importance in animals and man (Dubey 2010, Darwich et al. 2012, Nazir et al. 2018). Birds are susceptible to *T. gondii*, *N. caninum* and *Sarcocystis* spp. infection and they are considered intermediate hosts in these protozoa life cycles, consequently birds are important sources for humans and other animals infection (Sato et al. 2020, Dubey et al. 2021).

T. gondii infection in birds was described (Ibrahim et al. 2018, Bachand et al. 2019), as well as *N. caninum* infection in birds (Costa et al. 2008, Gondim et al. 2010). *Sarcocystis* species also had been detected in birds however, birds role in *Sarcocystis* life cycle is not complete determined (Konradt et al. 2017). Birds feeding habits as consumption of carcass (carnivore birds) or consumption of food and water touching the soil make possible birds infection by ingesting protozoa cysts or oocysts (Dubey 2010, Darwich et al. 2012, Nardoni et al. 2019, Dubey et al. 2021 Sato et al. 2020).

The role of birds in protozoa life cycles and epidemiology remains unclear (Dubey & Jones 2008, Gondim et al. 2010, Sato et al. 2020). Besides that, studies showing the occurrence of *T. gondii*, *N. caninum* and *Sarcocystis* spp. in birds are scarce (Darwich et al. 2012, Konradt et al. 2017, Nazir et al. 2018). Therefore, the importance of domestic and wild birds in protozoa epidemiology needs more attention. So, the objective of this work was the molecular detection of *T. gondii*, *N. caninum* and *Sarcocystis* spp. in diverse species of naturally infected wild and domestic birds.

MATERIALS AND METHODS

Samples

Birds who died from different causes and were destined to necropsy at the "Laboratório Central de Diagnóstico de Patologias Aviárias" (Avian Pathologies Diagnosis Central Laboratory - LCDPA) of the "Universidade Federal de Santa Maria" (UFSM) they were separated and analyzed. In study, were used tissue samples of brains and hearts from 65 birds, stored at -20°C until use DNA extraction is performed. The birds were classified in 33 species, belonging to 15 families (Accipitridae, Anatidae, Cacatuidae, Ciconiidae, Columbidae, Cracidae, Estrildidae, Fringillidae, Hirundinidae, Phasianidae, Psittacidae, Ramphastidae, Thraupidae, Turdidae and Tyranne) (Sick 1997). Birds were free-living, domestic or originating from conservatories or maintainer located in the city of Santa Maria, Rio Grande do Sul, Brazil (Table 1). Although the analyzed birds were inhabitants of places belonging to the city of Santa Maria, it is not possible to say that they lived all their lives in the same place because they have an unknown life history.

DNA extraction

A total of 130 fresh tissue samples (65 brains and 65 hearts) from birds were submitted to DNA extraction. DNA was extracted from 50mg of tissue using commercial kit (Wizard® Genomic DNA Purification Kit-Promega), following manufacturer instructions with one modification, the lysis step was performed at 55°C overnight, according to Moré et al. (2011). After extraction, DNA concentration was evaluated in all samples by measuring absorbance at 260nm with ultraviolet light (NanoDrop 1000, ThermoScientific, USA) and DNA concentration of all samples was adjusted to approximately 100ng/ul. After that, DNA samples were stored at -20°C until use.

Molecular detection

The diagnosis of infection by the protozoa *Toxoplasma gondii*, *Neospora caninum* and *Sarcocystis* spp. was carried out by detecting nucleic acids (DNA) using the polymerase chain reaction technique (PCR).

***T. gondii* PCR.** Extracted DNA from birds tissues was submitted to PCR amplifying repetitive gene 529 base pares for *T. gondii* using primers TOX4 (CGCTGCAGGGAGGAAGACGAAAGTTG) and TOX5 (CGCTGCAGACACAGTGCATCTGGATT) were selected from the 5' and 3' for amplification of a 529 bp fragment (Homan et al. 2000). Each PCR was performed in a total volume of 25µL, containing 2.5µL of10X buffer (Promega, USA); 0.65mM dNTPs (Ludwig Biotec, Brazil); 0.6µm of each primer (Sigma-Aldrich, Brazil); 1U Taq DNA Polymerase (Promega, USA); 1.25mM MgCl and 100ng of DNA as template. DNA extracted from tachizoites of *T. gondii* RH strain was used as positive control and MilliQ water was used as negative control. The PCR was carried out using a T100 thermal cycler (BioRad, USA) under the following conditions: 10 min at 95°C for the initial hot denaturation step, followed by 30 cycles of 30 s at 94°C, 1 min at 65°C, 1 min at 72°C, and a final extension step of 5 min at 72°C. The PCR products were visualized by UV illumination after electrophoresis at 1% agarose gel stained with Gel Red Nucleic Acid Stain (Biotium, USA).

***N. caninum* PCR.** PCR was performed using a set of primers Np6 (CAGTCAACCTACGTCTTC) and Np21 (GTGCGTCCAATCCTGTAA) amplifying NC5 specific gene, sequence primers 5' and 3' amplifying a fragment of 328 bp (Yamage et al. 1996). Each reaction was made

in a final volume of 25µL, containing 2.5µL of 10X buffer, 0.5µM of each primer, 0.5mM deoxynucleotide triphosphate (dNTPs) (Kapa, Bio Systems, Boston/MA, USA), 1U of Taq DNA Polymerase GoTaq® (Hot Start Polymerase, Promega, Madison/WI, USA), 0.75mM MgCl and 100ng of DNA. DNA extracted from tachizoites of *N. caninum* NC1 strain was used as positive control and MilliQ water was used as negative control. The PCR was carried out using a T100 thermal cycler (BioRad, USA) under the following conditions: 5 min at 95°C for the initial hot denaturation step, followed by 35 cycles of 1 min at 95°C, 1 min at 50°C, 1 min at 72°C, and a final extension step of 10 min at 72°C. The PCR products were visualized by UV illumination after electrophoresis at 1% agarose gel stained with Gel Red Nucleic Acid Stain (Biotium, USA).

Table 1. Classification of the 65 birds in families, species and housing in the municipality of Santa Maria, Rio Grande do Sul, Brazil

Family	Scientific name	Number of animals	Housing
Accipitridae	*Rupornis magnirostris*	1	Maintainer
Anatidae	*Dendrocygna bicolor*	1	Maintainer
Cacatuidae	*Nymphicus hollandicus*	2	Domestic
Ciconiidae	*Ciconia maguari*	1	Wild
Columbidae	*Columbina passerina*	1	Wild
Columbidae	*Leptotila verreauxi*	1	Wild
Columbidae	*Zenaida auriculata*	2	Wild
Cracidae	*Crax fasciolata*	2	Maintainer
Estrildidae	*Erythrura gouldiae*	1	Conservatory
Estrildidae	*Poephila cincta*	2	Conservatory
Estrildidae	*Taeniopygia guttata*	1	Conservatory
Fringillidae	*Serinus canaria*	3	Conservatory
Hirundinidae	*Pygochelidon cyanoleuca*	1	Wild
Phasianidae	*Chrysolophus pictus*	1	Conservatory
Phasianidae	*Lophura swinhoii*	1	Conservatory
Phasianidae	*Pavo cristatus*	1	Conservatory
Phasianidae	*Syrmaticus ellioti*	1	Conservatory
Psittacidae	*Amazona aestiva*	7	Maintainer
Psittacidae	*Amazona aestiva*	1	Conservatory
Psittacidae	*Amazona amazonica*	1	Maintainer
Psittacidae	*Amazona pretrei*	1	Conservatory
Psittacidae	*Amazona vinacea*	1	Maintainer
Psittacidae	*Ara chloropterus*	1	Maintainer
Psittacidae	*Melopsittacus undulatus*	2	Domestic
Psittacidae	*Myiopsitta monachus*	1	Domestic
Psittacidae	*Myiopsitta monachus*	1	Maintainer
Psittacidae	*Neophema splendida*	1	Conservatory
Psittacidae	*Neopsephotus bourkii*	5	Conservatory
Psittacidae	*Platycercus eximius*	6	Conservatory
Psittacidae	*Forpus xanthopterygius*	1	Conservatory
Psittacidae	*Psephotus haematonotus*	2	Conservatory
Psittacidae	*Psephotus haematonotus*	1	Conservatory
Ramphastidae	*Ramphastos dicolorus*	3	Maintainer
Thraupidae	*Paroaria coronata*	5	Maintainer
Turdidae	*Turdus rufiventris*	1	Wild
Tyrannidae	*Tyrannus savana*	1	Wild
15	33	65	

***Sarcocystis* spp. PCR.** Detection of *Sarcocystis* spp. by PCR amplifying the 18S rRNA region of the gene was carried out using 2L (GGATAAACCGTGGTAATTCTATG) and 3H (GGCAAATGCTTTCGCAGTAG), sequence primers 5' and 3'amplifying a 900 bp fragment (Yang et al. 2001). Each PCR was performed in a total volume of 25µL, containing 3µL of 10X buffer (Promega, USA); 1mM dNTPs (Ludwig Biotec, Brazil); 1.2uM of each primer (Sigma-Aldrich, Brazil); 1U Taq DNA polymerase (Promega, USA); 1.5mM MgCl and 100ng of DNA as template. DNA extracted from a pool of 50 cysts of *Sarcocystis* spp. was used as positive control and MilliQ water was used as negative control. The PCR was carried out using a T100 thermal cycler (BioRad, USA) under the following conditions: 2 min at 95°C for the initial hot denaturation step, followed by 40 cycles of 40 s at 94°C, 50 s at 56°C, 6 s at 72°C, and a final extension step of 6 min at 72°C. The PCR products were visualized by UV illumination after electrophoresis at 1% agarose gel stained with Gel Red Nucleic Acid Stain (Biotium, USA).

PCR inhibition test

Additional test was performed to determine whether PCR amplification may fail due the presence of PCR inhibitors in the samples.

Mixing positive and negative DNA templates. A possible effect of PCR inhibitors that could be present together with DNA templates which did not resulted in *N. caninum*, *Sarcocystis* spp. or *T. gondii* DNA amplification was tested by mixing positive control DNA templates with negative samples extracted from birds brain or heart fragments. Therefore, samples control positive were mixed 1:1; 5/5µL with negative samples (i.e. 5 brain samples and 5 heart samples for each parasite analyzed) and submitted to same PCR conditions.

Gene sequencing

Positive PCR products for *N. caninum* and *Sarcocystis* spp. were purified using QIAquick® PCR Purificatoin Kit (Qiagen™, Germany) according to manufacturer instructions. Final purified DNA was analyzed using spectrophotometer NanoDrop 1000 (ThermoScientific, USA) for concentration determination. After PCR purification, the sequencing reactions were performed using 5pmol of primers separately, 30-60ng of purified PCR product and MiliQ water in a final volume of 6µL. Followed by dehydration at 60°C for 2 hours and finally submitted to sequencing (ACTGENE - Serviço de Sequenciamento, Brazil). The results obtained were analyzed using StandenPackage software and the generated nucleotides sequences evaluated in Genbank NCBI database blast search[4].

Gene identification and phylogenetic analysis

Phylogenetic analyses were conducted separately for the 18S rRNA by using MEGA X software. Sequence analyses were performed with the BLASTX software[5]. Nucleotide sequences were aligned and compared to sequences from various hosts were downloaded from GenBank database using ClustalW. The alignments were optimized with the BioEdit Sequence Alignment Editor Program version 7.2. Phylogenetic analysis was carried out in MEGA-X, using the maximum likelihood. The confidence of each branch in the phylogeny was estimated with bootstrap values calculated from 1000 replicates.

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[4] Available at <http://www.ncbi.nlm.nih.gov/ BLAST> Accessed on Apr. 6, 2020.

[5] Available at <http://www.ncbi.nlm.nih.gov/blast/> Accessed on Aug. 20, 2020.

Authorization for use tissue samples of dead bird

This study was authorized by "Instituto Chico Mendes de Conservação da Biodiversidade" (Chico Mendes Institute for Biodiversity Conservation - ICMBio) under the "Sistema de Autorização e Informação em Biodiversidade" (Biodiversity Authorization and Information System - SISBIO) registration number 76022-1. Animals' Use Ethics Committee (CEUA) registration number 8263100820 because the study involves only dead animals not necessary approval.

RESULTS

In the study, DNA from *Toxoplasma gondii* was not detected in tissue sample, in addition to DNA from *Neospora caninum* or *Sarcocystis* spp. was found in five birds (05/65, 7.69%) (Table 2). *N. caninum* DNA was detected by PCR and sequencing confirmed in two birds, in brain tissue of a *Rupornis magnisrostris* (accession number: ON182081, 267pb) and in brain and heart tissues of a *Dendrocygna bicolor* (accession number: ON211312, 267pb) (Table 2).

Sarcocystis spp. DNA was detected by PCR in three birds: in brain tissue of a *Nymphicus hollandicus*, in brain and heart of *Amazona aestiva* and in brain and heart of a *Paroaria coronata* (Table 2). The samples tissue of the three birds were sequenced, but unsuccessful for brain and heart samples of a *Paroaria coronata*. Genetic sequencing confirmed and were sequences deposited in GenBank the presence of *Sarcocystis* spp. in the brain of *Nymphicus hollandicus* (accession number: MW463929, 712pb) and *Sarcocystis speeri* in the brain and heart of a *Amazona aestiva* (accession number: MW464125, 650pb). No mixed infections were observed.

Phylogenetic analysis based of the 18S rRNA (Fig.1) revealed that *Sarcocystis* spp. formed a clade with *Sarcocystis* spp. that utilize *Didelphis aurita* as the known or presumed definitive host and *Sarcocystis falcatula* that utilize *Trichoglossus moluccanus* as intermediate host. *Sarcocystis speeri* formed a clade with *S. speeri* that utilized *Mus musculus* (gamma interferon gene knockout mice) as intermediate host in bioassay. Besides, formed a clade with *Sarcocystis columbae*, *Sarcocystis corvusi*, *Sarcocystis halieti* and *Sarcocystis* sp. that affect outers species birds. Additional test was performed to evaluate whether PCR inhibitors could be influencing negative results and all of there-tested samples (10 previously negative samples mixed with *T. gondii* DNA – 10 previously negative samples mixed with *N. caninum* DNA – 10 previously negative samples mixed with *Sarcocystis* spp. DNA) resulted in positive both parasites DNA amplification indicating the absence of inhibitors in PCR reaction.

DISCUSSION

DNA from *Toxoplasma gondii* was not detected in tissue sample. Although birds are more resistant to *T. gondii* infection, DNA of *T. gondii* has already been detected in birds tissues in different countries (Gondim et al. 2010, Lukášová et al. 2018). Birds eating habits involve direct contact with soil and water potentially contaminated with sporulated oocysts, which is considered the most important form of *T. gondii* infection for animals (Dubey 2010). Therefore, the absence of *T. gondii* DNA in the analyzed birds samples was unexpected due the wide environmental contamination with *T. gondii* (Dubey et al. 2021).

DNA detection suggests that there were infection of *Neospora caninum* in wild birds, demonstrating that the positive detected birds were susceptibility to *N. caninum* infection and that birds were naturally infected. However, infection in birds has been described by different authors and *N. caninum* DNA had already been detected in sparrows tissues in Brazil (Costa et al. 2008, Gondim et al. 2010) and in other places in the world as in woodpeckers and vultures in Spain (Darwich et al. 2012), in crows in Israel (Salant et al. 2015) and pigeons in China (Du et al. 2015). A study demonstrated a higher prevalence of Neosporosis in cattle in farms that have the presence of birds (Darwich et al. 2012). *N. caninum* infection showed in the present study reinforce birds as an intermediate host and consequently serving as a source of infection for definitive host.

DNA from *Sarcocystis* spp. was detected in the species *Nymphicus hollandicus* and *Paroaria coronata.* Nucleic acids of *Sarcocystis speeri* was detected in *Amazona aestiva.* In a Brazilian zoo described *Sarcocystis* of the outbreak involving parakeets, cockatoos, parrots and pigeons (Ecco et al. 2008). In South American, other species have been described, such *Sarcocystis falcatula*-like (Dubey et al. 2000) and *Sarcocystis lindsayi* (Dubey et al. 2001).

S. speeri was described and named by Dubey & Lindsay (1999), who used an experimental intermediate host (*Mus musculus*) susceptible to infection, but the natural intermediate hosts are unknown. *Didelphis albiventris* and *Didelphis virginiana* are considered definitive hosts for *S. speeri* (Dubey et al. 2000). Besides, *S. falcatula* and *Sarcocystis neurona* use *Didelphis* spp. as definitive hosts (Gondim et al. 2021). In South America may act as definitive hosts *D. albiventris*, *Didelphis aurita*, *Didelphis marsupialis*, *Didelphis imperfecta* and *Didelphis pernigra*. (Cerqueira 1985, Lemos & Cerqueira 2002). Studies report that *S. speeri* and *S. neurona* are not infective to *Melopsittacus undulatus*, but are infective to

Table 2. *Toxoplasma gondii, Neospora caninum* and *Sarcocystis* spp. nucleic acid detection in brain and heart of naturally infected birds

Scientific name	Number of animals tested	Number of animals positive	*Toxoplasma gondii*		*Neospora caninum* (2/65 - 3.07%)		*Sarcocystis* spp. (3/65 - 4.62%)	
			Heart	Brain	Heart	Brain	Heart	Brain
Rupornis magnirostris	1	1	-	-	-	1	-	-
Dendrocygna bicolor	1	1	-	-	1	1	-	-
Nymphicus hollandicus	2	1	-	-	-	-	-	1
Amazona aestiva	7	1	-	-	-	-	1	1
Paroaria coronata	5	1	-	-	-	-	1	1
TOTAL		5/65 (7.69%)	0	0	1/65 (1.54%)	2/65 (3.07%)	2/65 (3.07%)	3/65 (4.62%)

gamma interferon gene knockout (KO) mice and, species *S. falcatula* and *S. lindsayi* are infective to *M. undulatus* but not to KO mice (Dubey et al. 2016).

In general, the *N. hollandicus*, *A. aestiva* and *P. coronata* are classified as a granivorous birds and under natural conditions its diet consists of a wide variety of seeds, fruits, leaves, flowers and insects (Sick 1997). The presence of DNA of parasites of genre *Sarcocystis*, in the present study, suggests these birds as intermediate host and also deduces that probably became infected by ingestion of contaminated water or food containing oocysts or *Sarcocystis.* Omnivorous or carnivorous birds have been identified as definitive hosts for *Sarcocystis* (Valadas et al. 2016).

The detection of *Sarcocystis* spp. and *S. speeri* in tissue samples from birds contribute to the extension of intermediate hosts. *Sarcocystis* spp. present a wide range of both definitive and intermediate hosts, and their detection in bird samples is extremely relevant, since birds are prey in the food chain of definitive hosts. Additionally, birds infected with *S. speeri* may be a risk factor for *D. albiventris* and *D. virginiana* infection when they ingest infected bird carcasses containing tissue cysts (Dubey & Lindsay 1999).

Diverse animal species are involved in *Sarcocystis* life cycle contributing to the wide protozoa distribution and previous studies had already demonstrated the presence of *Sarcocystis* in wild birds tissues (Prakas & Butkauskas 2012). Therefore, DNA detection *N. caninum*, *Sarcocystis* spp. and *S. speeri* reinforce protozoa infection potential in wild and domestic birds species and the importance of birds in protozoa epidemiology.

CONCLUSIONS

DNA of *Neospora* or *Sarcocystis* was detected in five birds. *Neospora caninum* nuclei acids were detected in tissue samples of naturally infected *Rupornis magnirostris* and *Dendrocygna bicolor*. *Sarcocystis* spp. DNA was detected in tissue samples *Nymphicus hollandicus* and *Paroaria coronata* and *Sarcocystis speeri* DNA was detected in tissue samples *Amazona aestiva* of naturally infected.

This seems to be the first report of DNA detection of *N. caninum*, *Sarcocystis* spp. and *S. speeri* in tissue samples to these birds species extending the list of intermediate hosts. Considering there is a great diversity of domestic and wild birds, studies that investigate their infection by protozoa are very relevant, since they clarify which species are host to the protozoa.

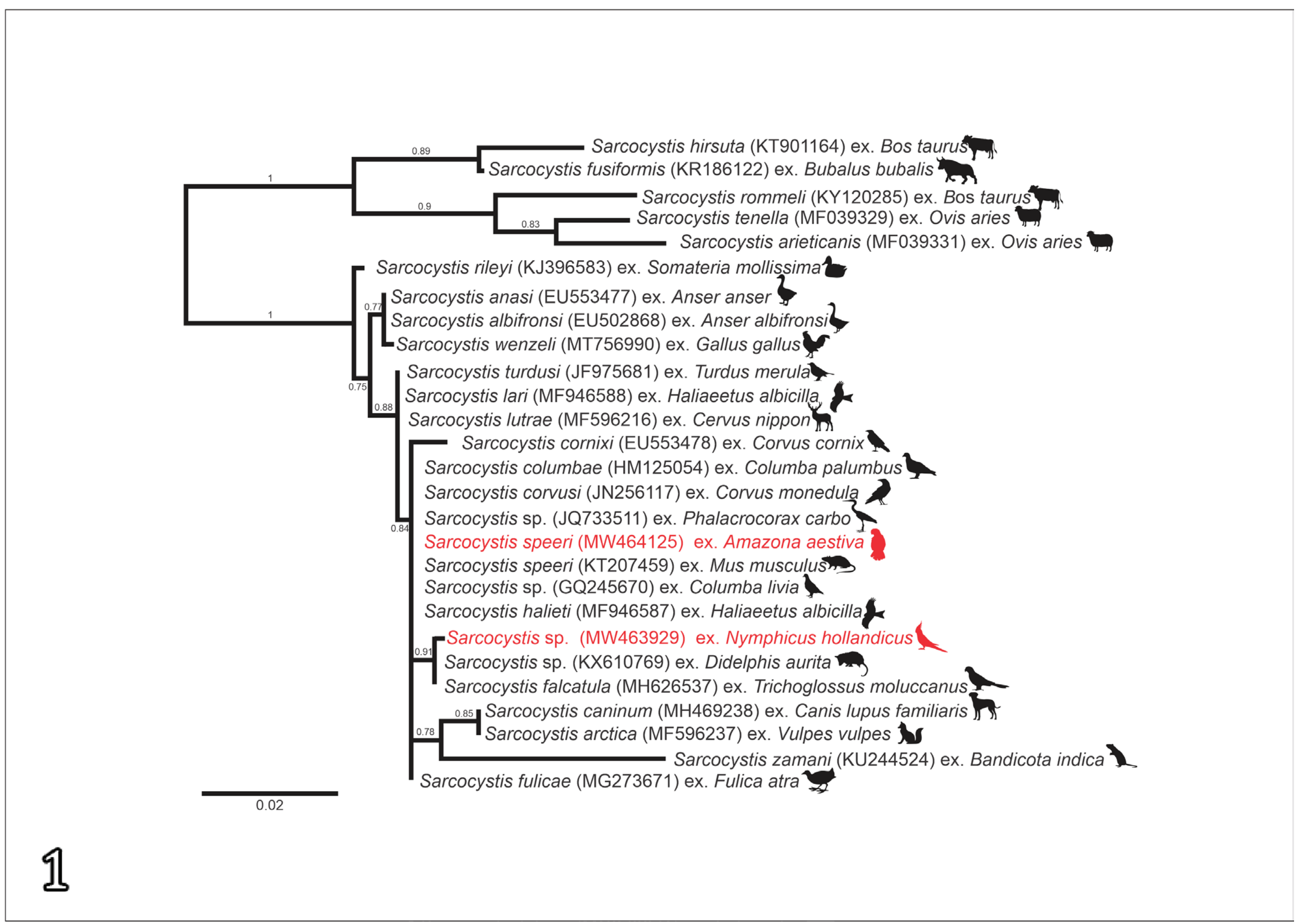

Fig.1. Phylogenetic analysis of 18S rRNA sequences obtained from various hosts compared to representative members of Sacocystidae family. The values between the branches represent the percent bootstrap value per 1000 replicates, and values below 70% are not shown.

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Conflict of interest statement.- The authors have no conflicting interests.

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