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Hemogregarine parasites in wild captive animals, a broad study in São Paulo Zoo

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Abstract
Hemogregarine is a group of blood parasites that infect a wide variety of vertebrates and hematophagous invertebrates. The signs of infection can range from anemia to severe interference in host's fitness. The purpose of this study was to gather information from the database available at the Clinical Analyses Laboratory at São Paulo Zoo Foundation in the last ten years and determine the occurrence of hemogregarine parasites in captive animals of the São Paulo Zoo Foundation. The analysis was conducted on the haemoparasitic results from 2972 blood samples, of 1637 individuals of all terrestrial vertebrate group (mammals, birds, reptiles and amphibians). Positive results were observed in 1.1% of the individuals and this parasite was found only in reptiles and amphibians. The lack of study with hemogregarine parasites infecting reptiles and amphibians is evident; this work will contribute to the knowledge of parasitological data for captive animals in future works.

Keywords: haemoparasites, *Hepatozoon*, reptile, amphibian, Apicomplexa

1. Introduction
Apicomplexan parasites from Adeleina suborder are recognized as hemogregarine and are composed of six genera: *Cyrilia*, *Desseria*, *Haemogregarina*, *Hemolivia*, *Hepatozoon*, and *Karyolysus* [1-3]. *Cyrilia* and *Desseria* are known as fish parasites and are transmitted by leeches [1, 4]. *Haemogregarina* can be found in aquatic and terrestrial hosts, such as fishes, turtles, tortoises and amphibians, being transmitted by leeches, mosquitoes and ticks [1, 5]. *Hemolivia* has only three species described so far, the reports consisting of infections in amphibians and tortoises, being transmitted by ticks, and is the only hemogregarine parasite that has erythrocyte merogony [6]. The most known parasite of this group is *Hepatozoon*, which has been reported in mammals, birds, amphibians and reptiles and can be transmitted by ticks and mosquitoes or by ingestion of another infected vertebrate host [2, 3, 7]. *Karyolysus* has been reported in lizards from Europe and Asia and has as the only vector the mite *Ophionyssus* sp. [8].
Traditionally, studies that identified these parasites used only erythrocytic stage of development, and these descriptions are based on the measure of the length and width of gamont and its nucleus [3, 7, 9]. Despite that, differentiating the parasite genera using only morphological features of blood stages can be difficult and it is not recommended by many authors [1, 2, 10]. Nevertheless, parasites' morphology can vary according to the technique used to made blood smears [8], and is already known, for other intraerythrocytic parasites, that they can have some morphological variations depending on their hosts [11]. To describe new species and identify parasite genus it is recommended to combine data of its ultrastructural features, blood, tissue and vector development stages [1, 2, 9].
Even, there is few data about clinical symptoms for five of six genera of these parasites; for *Hepatozoon* they are better known, especially for mammals [12]. Notwithstanding the fact that infections in wild animals are usually subclinical [13, 14], some symptoms have been associated with anaemia, erythrocyte hypertrophy, haemoglobin loss and alterations in the plasma membrane of erythrocytes [15]. Although the lack of information about the effects of hemogregarine infections in their hosts, it is well known that parasites can influence the fitness, life cycle and even reproduction and offspring survival rates [16-18].
Zoos and aquariums have the role to promote a clear view of the contribution that they can make as stakeholders of the global conservation efforts [19].

So, taking this into consideration, the objective of this work was to gather the information available in the database of the clinical analyses laboratory during the last nine years and report the occurrence of hemogregarine parasites in captive wild animals from São Paulo Zoo Foundation.

2. Materials and methods

2.1 Study area

São Paulo Zoo Foundation (SPZF) is located inside the Parque Estadual das Fontes do Ipiranga, one of the most important remaining fragments of the Atlantic Rain Forest in São Paulo City that harbors some of the Ipiranga stream and shelter dozens of native species. Since 1958, SPZF provides interaction with nature and technical professional training, and lately, has been intensely involved in scientific research, integrated actions for *ex situ* and *in situ* conservation of wildlife, effective participation in national plans for maintenance and reproduction of endangered native species allied to education programs to promote citizenship and conscience on environmental awareness [20].

2.2 Population studied and sample preparation and analysis

This study conducted a retrospective analysis of hemogregarine parasites in wild captive animals using the results of exams of 2972 blood samples collected from 1637 individuals, including 639 avian, 616 mammals, 375 reptiles, and seven amphibians. The avian group is represented by 130 species of 26 different families (Table 1); mammals by 96 species and 32 families (Table 2); reptiles by 66 species of 21 families (Table 3); and amphibian by four species and three families (Table 4). All exam that presented positive results for hemogregarine or *Hepatozoon* sp. were considered positive for this study.

The samples were collected during the veterinary routine for preventive or emergency exams and sent to the Clinical Analyses Laboratory at SPZF, in the period of April 2007 to May 2016. Laboratory already has a protocol for blood collection and processing, blood smears are prepared with or without the anticoagulants (EDTA or lithium heparin); air dried and stained using Rosenfeld technique [21] for reptiles, amphibians and birds, while mammals had their smears stained by a rapid stain Instant-Prov (New Prov). Each blood smear has 100 field examined under low magnification (400X) and in oil immersion (1000X) [22].

3. Results

Hemogregarine infections were found in 0.8% of evaluated samples (23/2972). The positive cases represented 1.1% of all individuals sampled (18/1637), but only reptiles and amphibians were positive (Table 5). The positive results represented 3% reptiles sampled (20/672) and 42.8% of amphibians sampled (3/7). The parasite was present in 18 individuals: one lizard, three amphibians and 14 snakes (Table 6).

The parasite was found in seven species of snakes (*Hydrodynastes gigas, Eunectes murinus, Epicrates chenchria, Corallus caninus, Boa constrictor, Spilotes pullatus, Corallus hortulanus*), one species of lizard (*Varanus doreanus*) and in two species of amphibians (*Rhinella marina, Rhinella schneideri*). All birds and mammals that had their results analyzed were negative for hemogregarine infections.

4. Discussion

The analysis of results of the microscopic examination to detect the presence of hemogregarine parasites in blood smears of captive wild animals was conducted at the Laboratory of Clinical Analyses of SPZF, using samples processed during the last ten years, and it is one of the largest studies done in this field.

It is known that Hemogregarine parasites have very similar morphological characteristics, being hard to differentiate them in their blood stage using only microscopic analysis [1, 2]. These parasites infects erythrocytes which can be deformed during blood smears preparation, affecting parasite shape if this procedure is not properly done [8, 23]. On the other hand, prior studies have suggested that hemogregarines found in reptiles and birds should be referred as *Hepatozoon* [1, 3]. For that reason, it is suggested that hemogregarine found in this study belong to genus *Hepatozoon.*

This study had seven species of snakes with infection by hemogregarine infections, all of them had already been described harboring these parasites [24, 25]. The only positive lizard was *Varanus doreanus*, but there is no report of *Hepatozoon* spp. infections in this host.

The prevalence of infections found in this study (1.1%) is lower than infections found by *Hepatozoon* sp., the most common hemoparasite found in reptiles [7], with the prevalence ranging from 8% to 48% [26-29]. Among the reptiles that have been described as infected with this parasite, the most common are *Boa constrictor* [30-32] and *Crotalus durissus* [32-34]. Besides the varieties of *Hepatozoon* spp. found in snakes, it is worth to highlight the presence of this parasite in *Caiman c. crocodilus* [35], *Gecko gecko* [36] and tuataras species [37], in the present study there was no infections by hemogregarine in any of *Caiman* sp. or *Gecko* sp.

In the amphibians group, the parasite was found in two species of toads, there was not was found any literature report for *Hepatozoon* spp. infections in the toad species that were positive in this study; despite of that, these infections should be more carefully investigated to confirm the hemogregarine species involved in the infections. The prevalence of infections in amphibians was of 42.8% in this study, which corroborates with the literature that shows prevalence in amphibian ranging between 2.6% to 75.4% and can vary seasonally over spring and winter [38-40]. *Hepatozoon* spp. were already reported infecting mainly *Rana catesbeiana* [35, 39, 41, 42], *Rana clamitans* [39, 41-44], *Rana pipiens* [42, 45]; but can also infects *Seychelles caecilians* [46], *Amietia quecketti* [40], *Leptopelis christyi, Leptopelis kivuensis, Ptychadena mascareniensis* [47], *Leptodactylus chaquensis, Leptodactylus podicipinus* [38], *Rana pretiosa* [48] and *Hoplobatrachus rugulosus* [49], but these studies were carried out only in wildlife animals. About the body size of the amphibians studied, it is important to highlight the difficulty to obtain adequate blood sample volume for analysis in small animals, such as the body size of most captive amphibians at SPZF. For this reason, this data does not represent a characteristic of our collection and they should be analyzed carefully.

There was not any positive bird species for hemogregarine infections in this study. *Hepatozoon* species found in birds are relatively uncommon and little is known about its life cycle, symptoms and virulence in infected animals [50]. Some authors described many species of *Hepatozoon* in birds [51-53], but the use of molecular techniques showed that some of them were from *Lankesteria* sp. and not *Hepatozoon* sp. Although the presence of *Hepatozoon* is rare in birds, the most part of findings was in water birds. Frequency of *Hepatozoon* sp. infections in *Oceanodroma* spp. was reported as 42.5% [54]; and the parasite was found in the freshwater birds

Acrocephalus arundinaceus and *Acrocephalus scirpaceus* [55]. A prevalence of 31.2% based on microscopy was also registered in *Cyanistes caeruleus* [56].

The presence of hemogregarine in mammals was not verified in the present study. Despite of that, they can infect a wide variety of mammals, mainly in canids and felids, and the study of the host-parasite relationship has increased in the past decade due to its veterinary importance [57]. A molecular survey realized in different Brazilian zoos with this parasite in captive canids, found *Hepatozoon* spp. in bush dogs in the same study site of the present study [58]. For Brazilian mammals, a *Hepatozoon* spp. prevalence of 78.2% was registered in *Cerdocyon thous* and 71.4% in *Leopardus pardalis* [59]. *Hepatozoon* was also detected in *Nasua nasua* and *Procyon cancrivorus* [60]. For exotic species of mammals, this parasite was found in five out of nine *Panthera leo*, two out of five *Panthera tigris tigris*, two out of four *Panthera pardus* and described a *Hepatozoon ursi* in *Melursus ursinus* [61, 62]. Some other studies show that *Hepatozoon* spp. can occur in *Vulpes vulpes* [63] and *Canis mesomelas* [64].

Around 45% of reptile and 85% of amphibians species had their conservation status evaluated by International Union for Conservation of Nature Redlist (IUCN) [65]; knowing the conservation status of positive animals is as important as know the fitness influence that hemogregarine parasites cause in their hosts, especially in wildlife conservation field. The IUCN classifies *Corallus caninus, Corallus hortulanus, Rhinella marina, Rhinella schneideri* and *Varanus doreanus* as least concern, while *Eunectes murinus, Spilotes pullatus* and *Boa constrictor* are classified as not threatened [65]. There is no data for *Hydrodynastes gigas* in IUCN Redlist, but is classified as endangered by the Instituto Chico Mendes de Conservação da Biodiversidade [66]. There is no data about the conservation status for *Epicrates chenchria.* All of the positive species are native from Brazil, except for *Varanus doreanus* that is native from Australia, Indonesia and Papua New Guinea [65].

Table 1: Species of birds sampled in this study

Specie	N	n
Acridotheres cristatellus	2	2
Alopochen aegyptiaca	11	8
Amazona aestiva	27	10
Amazona amazonica	3	3
Amazona brasiliensis	8	8
Amazona farinosa	5	3
Amazona festiva	9	7
Amazona ochrocephala	9	3
Amazona rhodocorytha	5	4
Amazona vinacea	10	7
Amazonetta brasiliensis	6	4
Anodorhynchus hyacinthinus	39	26
Anodorhynchus leari	25	15
Anser anser	1	1
Anser cygnoides	1	1
Ara ararauna	7	5
Ara chloropterus	6	3
Ara macao	11	4
Ara rubrogenys	5	5
Ara severus	2	2
Aramides cajaneus	1	1
Aratinga jandaya	6	2
Aratinga nenday	6	3
Asio stygius	4	3
Balearica regulorum	12	8
Branta canadensis	1	1
Brotogeris tirica	1	1
Bubo virginianus	3	1
Buceros rhinoceros	8	1
Bucorvus abyssinicus	11	4
Buteogallus coronatus	7	3
Buteogallus lacernulata	10	5
Buteogallus meridionalis	5	4
Buteogallus urubitinga	4	2
Cacatua alba	1	1
Cacatua galerita	6	2
Cacatua moluccensis	1	1
Cacatua s. citrinocristata	1	1
Caracara plancus	4	1
Cariama cristata	6	2
Cereopsis novaehollandiae	21	9
Chauna torquata	3	2
Chenonetta jubata	8	2
Chunga burmeisteri	14	5
Coscoroba coscoroba	20	18
Crax blumenbachii	1	1

Crax rubra rubra	1	1
Cyanopsitta spixii	4	2
Cygnus atratus	80	65
Cygnus melanocoryphus	28	19
Dendrocygna bicolor	4	1
Dendrocygna viduata	8	7
Deroptyus accipitrinus	4	2
Dromaius novaehollandiae	9	6
Egretta thula	1	1
Eos bornea	4	4
Eudocimus ruber	7	6
Eupsittula aurea	3	3
Falco femoralis	4	2
Geranoaetus albicaudatus	4	2
Geranoaetus melanoleucos	7	3
Graydidascalus brachyurus	1	1
Guaruba guaruba	10	6
Gypohierax angolensis	1	1
Haliaeetus vocifer	4	1
Harpia harpyja	17	10
Leptodon cayanensis	1	1
Megascops choliba	6	4
Milvago chimachima	2	1
Mitu tomentosun	6	4
Mitu tuberosun	1	1
Musophaga violacea	3	3
Netta erythrophthalma	2	2
Nothocrax urumutum	5	4
Nymphicus hollandicus	1	1
Odontophorus capueira	2	2
Pavo cristatus	52	35
Pavo muticus	1	1
Pelecanus onocrotalus	3	3
Penelope sp.	2	2
Phoeniconaias minor	2	2
Phoenicopterus chilensis	49	37
Pionites leucogaster	12	8
Pionopsitta pileata	1	1
Pionus fuscus	6	4
Pionus maximiliani	2	2
Pionus menstruus	9	4
Pipile jacutinga	6	3
Platalea ajaja	1	1
Plectropterus gambensis	6	4
Poicephalus senegalus	9	2
Primolius auricollis	3	3
Primolius maracana	5	2
Psarocolius decumanus	2	1
Pseudastur polionota	1	1
Pseudoscops clamator	15	3
Psittacara leucophthalmus	4	2
Psittacus erithacus erithacus	1	1
Pteroglossus aracari	17	5
Pteroglossus bailloni	9	4
Pteroglossus castanotis	2	2
Pteroglossus inscriptus	1	1
Pulsatrix koeniswaldiana	2	2
Pulsatrix perspicillata	9	4
Pyroderus scutatus	2	1
Pyrrhura frontalis	7	3
Pyrrhura lepida lepida	10	9
Pyrrhura perlata	4	2
Ramphastos dicolorus	32	7
Ramphastos toco	34	11
Ramphastos tucanus	28	5
Ramphastos vitellinus	16	3
Rhea americana	4	4
Rupicola rupicola	1	1
Sarcoramphus papa	16	8

Selenidera maculirostris	41	11
Spheniscus magellanicus	22	7
Spizaetus ornatus	24	10
Spizaetus tyrannus	12	6
Struthio camelus	20	15
Tadorna ferruginea	16	7
Tadorna radjah	8	2
Tadorna tadornoides	8	2
Tadorna variegata	2	1
Tauraco leucotis	5	4
Theatocercus acuticaudatus	3	2
Therisdicus caudatus	1	1
Trigonoceps occipitalis	9	1
Tyto alba	6	4
Vultur gryphus	2	1

N= number of samples; n= number of individuals.

Table 2: Species of mammals sampled in this study

Specie	**N**	**n**
Addax nasomaculatus	34	7
Aepyceros melampus	4	1
Alouatta caraya	15	3
Alouatta guariba clamitans	14	4
Ammotragus lervia	21	5
Aotus trivirgatus	4	2
Ateles chamek	4	4
Ateles marginatus	7	3
Ateles paniscus	6	3
Ateles sp.	25	15
Bison bonasus	13	7
Brachyteles arachnoides	10	3
Bradypus variegatus	1	1
Callithrix jacchus	2	2
Callithrix penicillata	1	1
Camelus bactrianus	8	5
Camelus dromedarius	3	3
Canis lupus	16	5
Caracal caracal	4	2
Cebus kaapori	4	3
Cebus olivaceus	10	5
Ceratotherium simum	4	1
Cerdocyon thous	40	8
Cervus elaphus	27	11
Choloepus didactylus	9	3
Choloepus hoffmanni	2	1
Chrysocyon brachyurus	28	6
Cuniculas paca	1	1
Dama dama	70	37
Dasyprocta azarae	1	1
Dasypus novemcinctus	3	1
Didelphis aurita	3	2
Eira barbara	13	4
Elephas maximus	3	2
Equus grevyi	6	4
Eudorcas thompsonii	2	2
Galictis vittata	3	3
Giraffa camelopardalis	7	4
Hippopotamus amphibius	2	2
Hylobates lar	2	1
Kobus e. ellipsiprymnus	22	13
Lagothrix lagotricha	14	4
Lama glama	11	6
Lama pacos	4	2
Leontopithecus chrysomelas	102	59
Leontopithecus chrysopygus	62	27
Leontopithecus rosalia	10	4
Leopardus colocolo	14	9
Leopardus geoffroyi	36	14
Leopardus pardalis	11	4

Leopardus tigrinus	113	40
Leopardus wiedii	29	11
Leptailurus serval	50	9
Lontra longicaudis	4	3
Lycaon pictus	2	2
Mazama americana	1	1
Mazama gouazoubira	7	2
Mico argentata	5	3
Myrmecophaga tridactyla	25	13
Nasua nasua	23	7
Oryx gazella	7	3
Otaria flavescens	2	2
Pan troglodytes	21	9
Panthera leo	46	14
Panthera onca	21	7
Panthera pardus melas	1	1
Panthera tigris altaica	28	11
Panthera tigris tigris	9	4
Panthera uncia	1	1
Papio cynocephalus	1	1
Phacochoerus africanus	2	2
Pithecia albicans	8	2
Pithecia pithecia	4	3
Pongo pygmaeus	8	2
Potos flavus	5	2
Prionailurus viverrinus	2	1
Puma concolor	8	4
Puma yagouaroundi	48	12
Rusa unicolor	11	7
Saguinus midas niger	11	4
Saimiri sciureus	6	3
Sapajus apella	43	29
Sapajus flavius	8	5
Sapajus xanthosternos	24	13
Speothos venaticus	7	4
Sphiggurus villosus	8	2
Suricata suricatta	14	10
Tamandua tetradactyla	49	16
Tapirus terrestris	16	5
Tayassu pecari	20	10
Tayassu tajacu	11	7
Tragelaphus strepsiceros	6	4
Tremarctos ornatus	3	2
Ursus americanus	3	2
Ursus arctos	2	1

N= number of samples; n= number of individuals.

Table 3: Species of reptiles sampled in this study

Specie	**N**	**n**
Aldabrachelis gigantea	10	4
Amphisbaena alba	2	2
Astrochelys radiata	1	1
Boa constrictor	22	11
Bothrops alternatus	7	4
Bothrops jararaca	5	4
Bothrops jararacussu	2	2
Bothrops moojeni	3	3
Caiman crocodilus	11	3
Caiman latirostris	33	11
Caiman yacare	3	1
Chelonoidis carbonarius	55	29
Chelonoidis denticulatus	38	22
Chelydra serpentina	25	7
Corallus caninus	4	3
Corallus hortulanus	12	4
Corucia zebrata	18	9
Crotalus durissus	4	4
Cuora amboinensis	2	1
Drymarchon corais	2	1

Epicrates cenchria	5	4
Epicrates crassus	3	2
Epicrates maurus	2	2
Erytrolamprus miliaris	5	3
Eryx colubrinus	2	2
Eublepharis macularius	3	3
Eunectes murinus	18	12
Graptemys pseudogeographica	2	1
Hydrodynastes gigas	5	3
Hydromedusa tectifera	1	1
Iguana iguana	41	13
Kinosternon scorpioides	13	9
Lampropeltis californiae	5	3
Lampropeltis getula	1	1
Melanosuchus niger	1	1
Mesoclemmys tuberculata	37	14
Mesoclemmys vanderhaegei	2	2
Morelia spilota	5	1
Oxyrhophus guibei	5	5
Paleosuchus palpebrosus	7	1
Paleosuchus trigonatus	7	2
Philodryas olfersii	1	1
Philodryas patagoniensis	4	4
Phrynops geoffroanus	13	8
Phrynops hillarii	10	10
Podocnemis concinna	1	1
Podocnemis expansa	20	18
Podocnemis unifilis	15	15
Pogona vitticeps	3	1
Pseudemys concinna floridana	4	3
Python curtus	2	1
Python molurus	1	1
Python regius	8	7
Salvator merianae	13	6
Spilotes pullatus	12	5
Stigmochelys pardalis	1	1
Testudo graeca	3	2
Tiliqua scincoides	8	3
Tomistoma schlegelli	5	2
Trachemys dorbigni	34	28
Trachemys scripta elegans	68	37
Trachemys scripta scripta	4	4
Tropidrodryas striaticeps	1	1
Tupinambis rufescens	7	2
Varanus doreanus	3	1
Xenodon merremi	2	2

N= number of samples; n= number of individuals.

Table 4: Species of amphibian sampled in this study

Specie	**N**	**n**
Leptodactylus labyrinthicus	3	3
Lithobates catesbeianus	1	1
Rhinella marina	2	2
Rhinella schneideri	1	1

N= number of samples; n= number of individuals.

Table 5: Prevalence of hemogregarine parasites in captive animals from São Paulo Zoo Foundation

	N (N+)	**P (%)**	**n (n+)**	**p (%)**
Amphibians	7 (3)	42.8	7 (3)	42.8
Birds	1152 (0)	0	639 (0)	0
Mammals	1141 (0)	0	616 (0)	0
Reptiles	672 (20)	3	375 (15)	4

N: number of samples; N+: number of positive samples; n: number of individuals; n+: number of positive individuals; P: prevalence of positive samples; p: prevalence of positive individuals.

Table 6: Positive species for hemogregarine parasites

Animal	N	N
Rhinella marina	2	2
Rhinella schneideri	1	1
Boa constrictor	1	1
Corallus caninus	2	2
Corallus hortulanus	2	2
Epicrates cenchria	3	3
Epicrates cenchria crassus	1	1
Eunectes murinus	1	1
Hydrodynastes gigas	1	1
Spilotes pullatus	6	3
Varanus doreanus	3	1

N= number of samples; n= number of individuals.

5. Conclusions

The role of hemogregarine parasites in wild animals is not completely understood, more studies addressing wild and captive animals should be done. The relevance of this study is supported by the considerable number of the samples and species that were analyzed, expecting that these findings would add a substantial contribution to the understanding on hemogregarine and their hosts, bringing more opportunities to improve studies and knowledge on this group of parasites. This study also provides a perception that training of professionals for conducting reliable microscopic analyses of diagnostic material is essential to maintain the good standards of laboratories devoted to recognition of parasitic evaluation of wildlife fauna. Considering that investigation and development of molecular techniques to pursue further studies is strongly recommended in this field, the Applied Research Department of São Paulo Zoo is currently establishing a molecular protocol for *Hepatozoon* spp. diagnosis to be used as a complementary technique to microscopic analyses of these species.

6. Acknowledgements

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