Parasitology Research (2019) 118:599–606
https://doi.org/10.1007/s00436-018-6155-5

PROTOZOOLOGY - ORIGINAL PAPER

Pathological and molecular characterization of avian malaria in captive Magellanic penguins (*Spheniscus magellanicus*) in South America

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Received: 7 May 2018 / Accepted: 12 November 2018 / Published online: 19 November 2018

Abstract

Avian malaria is a mosquito-borne disease that affects multiple avian species and is caused by protozoans of the genus *Plasmodium*. An avian malaria infection caused by *Plasmodium* sp. in Magellanic penguins (*Spheniscus magellanicus*) with high mortality is described in a zoo in Southern Brazil. Clinically, three birds presented signs of inappetence, anorexia, pale mucosa, dyspnea, and opisthotonus, with death in a clinical course of 5–8 h. At the necropsy, all birds exhibited pale mucosa, marked splenomegaly and hepatomegaly, in addition to moderate leptomeningeal blood vessels ingurgitation in the brain. Microscopically, multiple exoerythrocytic meronts were observed in the cytoplasm of endothelial cells in the spleen, liver, heart, lungs, brain, kidneys, and pancreas. The spleen had a multifocal perivascular inflammatory infiltrate of lymphocytes, plasma cells, and macrophages, which also exhibited hemosiderosis and erythrophagocytosis. The liver had a multifocal periportal inflammatory infiltrate of lymphocytes, macrophages, and plasma cells, in addition to marked hemosiderosis in the hepatic sinusoids. Fragments of spleen, liver, brain, skeletal muscle, and lung were tested by the polymerase chain reaction technique for the detection of a fragment of the cytochrome B gene from haemosporidians, which resulted positive for *Plasmodium* spp. After sequencing, the samples were phylogenetically associated to *Plasmodium* sp. detected in *Turdus albicollis* (KU562808) in Brazil and matched to the lineage TURALB01 previously detected in *T. albicollis.* Avian malaria infections caused by *Plasmodium* sp. of lineage TURALB01 may occur in *S. magellanicus* with high mortality, and, thus, it is essential to detect and characterize the agent involved to obtain the differential diagnosis of the condition.

Keywords Culicidae · Haemosporidian parasites · Morphologic analyses · Phylogenetic analyses · *Plasmodium* spp.

Introduction

Magellanic penguins (*Spheniscus magellanicus*) are native birds from Argentina, Chile, and Falkland Islands, and migrate to Southern Brazil from March to September. Annually, tens to hundreds of penguins, mainly juvenile, are admitted to rehabilitation centers in the Brazilian coast (García-Borboroglu et al. 2006, 2010). During this period, these animals may be affected by many diseases, such as aspergillosis, pododermatitis, and avian malaria (Cranfield et al. 1991).

Avian malaria is a mosquito-borne disease transmitted by blood feeding insects from family Culicidae that affects multiple bird species, and it is caused by protozoan of the genus *Plasmodium* (Valkiūnas 2005; Bueno et al. 2010; Sallaberry-Pincheira et al. 2015). Despite that, these parasites are not associated with events of mass mortality in bird populations who co-evolve with species of *Plasmodium* (Grilo et al. 2016). Many species of penguins are, however, highly susceptible to *Plasmodium* spp. both in the wild and in captivity (Vanstreels et al. 2016). Magellanic penguins of zoological gardens or undergoing rehabilitation, which are exposed for the first time to the agent are mainly affected (Fix et al. 1988;

Section Editor: Larissa Howe

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Cranfield et al. 1991; Bueno et al. 2010; Grilo et al. 2016), with infections caused by *Plasmodium relictum* (Fix et al. 1988), *Plasmodium elongatum*, *Plasmodium tejerai* (Silveira et al. 2013), *Plasmodium juxtanucleare* (Grim et al. 2003), *Plasmodium cathemerium* (Vanstreels et al. 2014), *Plasmodium nucleophilum*, and *Plasmodium unalis* (Vanstreels et al. 2015), while avian malaria has not been identified in free-living Magellanic penguins (Vanstreels et al. 2017).

Clinically, avian malaria is characterized by an acute course with anemia, lethargy, anorexia, fever, weight loss, and greenish feces, followed by high mortality indexes (Valkiūnas 2005). Pathological findings are predominantly related to the hemolytic disease and include hepatomegaly, splenomegaly, hydropericardium, and pulmonary edema (Fix et al. 1988). Microscopically, splenic and hepatic hemosiderosis are characteristic, with exoerythrocytic meronts inside endothelial cells and macrophages (Valkiūnas 2005). The present study aims to describe the clinical, pathological, and molecular aspects of avian malaria infection in Magellanic penguins (*S. magellanicus*), which culminated with substantial mortality at a zoo in the Southern Brazil.

Materials and methods

Three captive Magellanic penguins were kept in a zoo located in the city of Gramado, Rio Grande do Sul state, Brazil (29° 25′ 40″ S, 50° 51′ 18.8″ W), with an area of 20 ha that is surrounded by abundant secondary forests. Two adults (male and female; penguins 1 and 2), and a juvenile male (penguin 3) were clinically examined and died a few hours after the onset of clinical signs. The birds were housed in this zoo for at least 1 year and were previously admitted to a rehabilitation center located in the city of Florianópolis, Santa Catarina State (27° 31′ 40″ S, 48° 25′ 44″ W). Although these birds were kept isolated in an indoor enclosure with controlled temperature, they were fed at the outdoor enclosure where there was no mosquito control. Close to the penguin enclosure, there was an enclosure with various species of Psittaciformes (*Amazona* spp., *Ara* spp., and *Anodorhyncus hyacinthinus*).

The penguins were submitted to necropsy, when multiple fragments of tissues were collected, fixed in 10% neutral buffered formalin solution for 24–48 h, routinely processed for histology, and stained with hematoxylin and eosin (HE). No blood smears were available for evaluation in any of the cases.

Samples of spleen (penguins 1, 2, and 3), liver, brain, skeletal muscle (penguins 2 and 3), and lungs (penguin 1) were submitted to DNA extraction using the DNeasy® Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. All tissues were tested by nested-PCR for the presence of DNA of *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*, targeting mitochondrial cytochrome B (*cytB*), following the protocol previously described by Hellgreen et al. (2004). The first reaction was performed using the primers HaemNFI (5′-CATATATTAAGAGAAITATGGAG-3′) and HaemNR3 (5′-ATAGAAAGATAAGAAATACC ATTC-3′) in a final volume of 25 μL, which contained 50 ng of extracted DNA, 1.25 mM of each set of dNTP (Invitrogen, Carlsbad, USA), 1.5 mM $MgCl_2$, 10× concentrated buffer (Invitrogen®), 0.4 μM of each primer, and 0.5 U of Taq DNA polymerase (Invitrogen, Carlsbad, USA). Cycling conditions comprise an initial denaturation at 94 °C for 3 min, 20 cycles of 94 °C for 20s, 50 °C for 30s, 72 °C for 45 s, and a final extension at 72 °C for 10 min. Two microliters of the amplification products from the first reaction were employed in the subsequent nested PCR reactions: 1 μL for the PCR product for the detection of *Plasmodium* spp. and *Haemoproteus* spp. (using the primers HaemF 5′-ATGG TGCTTTCGATATATGCATG-3′ and HaemR2 5′-GCAT TATCTGGATGTGATAATGGT-3′), and 1 μL for the PCR product for the detection of *Leucocytozoon* spp. (using the primers HaemFL 5′-ATGGTGTTTTAGATACTTACATT-3′ and HaemR2L 5′-CATTATCTGGATGAGATAATGGIG C-3′). These reactions were performed separately in a final volume of 25 μL, under the same cycling condition and reagents concentration from the first reaction, except for 35 instead of 20 cycles. DNA samples obtained from an Orinoco goose (*Neochen jubata*) naturally infected with *Plasmodium* sp.—MF043229 (Werther et al. 2017) and ultra-pure sterilized water were used as positive and negative controls, respectively. The results were visualized in 2% agarose gel stained by ethidium bromide solution.

All the PCR products showing high intensity of the bands of expected sizes were purified using the Silica Bead DNA Gel Extraction Kit (Fermentas, São Paulo, Brazil). Purified amplified DNA fragments from each positive sample were sequenced in an automatic sequencer (ABI Prism 310 Genetic Analyzer; Applied Biosystems/Perkin Elmer, Waltham, USA) in both directions using Sanger's method (Sanger et al. 1977). The electropherogram quality was analyzed using the Phred Phrap software (Ewing et al. 1998), in which only nucleotide sequences above 400 bp in size and Phred quality ≥ 20 were used. Quality scores were assigned to each base call in automated sequencer traces. Additionally, consensus sequences were obtained through analysis on the sense and antisense sequences using the Phred Phrap software (Ewing et al. 1998). The identity values among the nucleotide sequences were assessed by BLASTn tool (using default parameters), available in MalAvi database (Bensch et al. 2009). The obtained sequences aligned to other sequences obtained from MalAvi database using the Mafft software (Katoh 2017) and were edited with the BioEdit software (Hall 1999). For phylogenetic tree reconstruction, a "best of it" evolution model was chosen using the jModelTest 2 software (Darriba et al. 2012). Bayesian analysis from the aligned matrices obtained

was performed with the MrBayes 3.2.2 in XSEDE software (Ronquist and Huelsenbeck 2003) available at the CIPRES portal (Miller et al. 2010), employing that the GTR + I + G evolution model, number of invariant sites (Pinvar) 0.5530, "gamma shape" 0.7320, nucleotide substitution model (Nucmodel) 4 × 4, number of generations (Ngen) 100.000, and Markov chain Monte Carlo (MCMC) simulations were run for 10^9 generations with a sampling frequency of every 100 generations with exclusion of 25% of the trees generated ("burn-in"). The phylogenetic tree edition and rooting (external group) were performed using the Treegraph 2.0 beta software (Stover and Muller 2010). *Haemoproteus noctuae*, *H. enucleator*, *H. tartakovskyi*, and *H. sanguinis* were used as outgroups.

Results

Over a course of 15 days in January 2017, all three Magellanic penguins housed at Gramado zoo exhibited, in distinct moments, peracute clinical signs of inappetence, anorexia, pale mucosa, dyspnea, followed by opisthotonus and death in a clinical course of 5–8 h. At necropsy, all birds had pale mucosa, enlarged firm dark-red spleen (three times its normal size), that bulged into the capsule on cut surface and had multifocal pinpoint white areas on the parenchyma (Fig. 1a). The liver was moderately (penguin 1) to severely (penguins 2 and 3) enlarged, occupying almost two thirds of the coelomic cavity, and showed rounded edges and multifocal irregular pale areas on the capsular surface (Fig. 1b). The leptomeningeal blood vessels of the brain were moderately distended (penguins 2 and 3) and the brain had a superficial cherry-pink discoloration (penguin 3). The lungs were diffusely pale and exhibited moderate deposition of a yellow material at the pleural surface (penguin 1), while the air sacs were mildly (penguin 2) to moderately (penguin 3) thickened.

Histologically, all penguins had in the cytoplasm of endothelial cells in the spleen, liver, heart, lungs, brain, kidneys, and pancreas multiple protozoa exoerythrocytic meronts delineated by a thin eosinophilic capsule. These exoerythrocytic meronts were frequently elongated and contained numerous randomly distributed basophilic merozoites, measuring 15–25 μm × 8 μm. Occasionally, these exoerythrocytic meronts were round to oval, measuring approximately 6–8 μm in diameter, with dozens of merozoites arranged concentrically in arcs or condensed, measuring approximately 1 μm in diameter each (Fig. 2a-b). Rarely, these exoerythrocytic meronts were observed inside macrophages in the spleen and liver.

The spleen had loss of distinction between the white and red pulp with a severe inflammatory infiltrate, frequently, perivascular of lymphocytes, plasma cells, and macrophages, which often exhibited a brownish pigment in the cytoplasm (hemosiderosis) and, occasionally, erythrophagocytosis (Fig. 2c). The liver had a marked multifocal periportal to perivascular inflammatory infiltrate of lymphocytes, macrophages, and occasional plasma cells (Fig. 2d). The hepatic sinusoids also showed large amounts of macrophages containing a brownish pigment in the cytoplasm (hemosiderosis). Extramedullary hematopoiesis was not observed in the liver. The lungs had moderate (penguin 1) to severe (penguins 2 and 3) interstitial inflammatory infiltrate of macrophages (interstitial pneumonia) (Fig. 2e). The bone marrow exhibited a marked hyperplasia of the myeloid lineage, in addition to a mild perivascular inflammatory infiltrate of lymphocytes and plasma cells (Fig. 2f). Table 1 summarizes the distribution of protozoa exoerythrocytic meronts in the different organs.

Nine out of the 10 tested samples were positive in the nested-PCR assay targeting *Plasmodium* spp. and *Haemoproteus* spp. (Table 2). Based on the intensity of the amplified bands, three amplicons were sequenced from penguins 2 and 3. Amplicons from penguin 1 could not be sequenced due to weak intensity of the amplified bands. All samples were uninfected to *Leucocytozoon* spp. according to nested PCR assays.

At BLASTn analysis, two haemosporidian *cytB* sequences obtained from brain and muscle samples of penguin 3 (accession numbers: MG602832, MG602833) showed 100% identity to a sequence of *Plasmodium* sp. detected in a white-

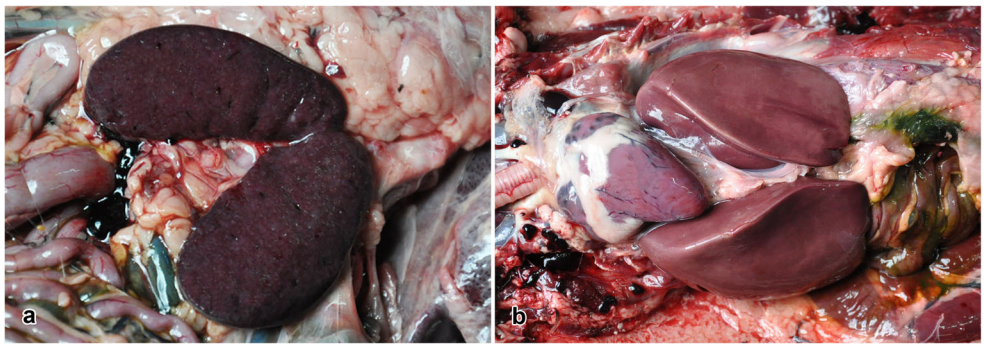

Fig. 1 Gross findings of *Plasmodium* sp. infection in captive Magellanic Penguins (*Spheniscus magellanicus*) in South America. **a** The spleen of penguin 2 was severely enlarged and had multifocal pinpoint white areas on the parenchyma on the cut surface. **b** The liver of penguin 2 was also severely enlarged with rounded edges and occupied two thirds of the coelomic cavity

Table 1 Exoerythrocytic meronts distribution in the organs of three Magellanic penguins (*Spheniscus magellanicus*)

Organ	Penguin 1	Penguin 2	Penguin 3
Spleen	+	+	+
Brain	+	+	+
Skeletal muscle	–	–	+
Lungs	+	+	+
Liver	+	+	+
Kidneys	+	+	+
Pancreas	+	+	+

+: presence of exoerythrocytic meronts; −: absence

necked thrush (*Turdus albicollis*) sampled in the Brazilian Amazon (KU562808) and 99% identity to a sequence of *Plasmodium* sp. detected in a creamy-bellied thrush (*Turdus amaurochalinus*) also sampled in the Brazilian Amazon (KU562569). On the other hand, the *cytB* sequence obtained from the brain sample of penguin 2 (accession numbers: MG602834) showed 100% identity to a sequence of *Plasmodium* sp. detected in a white-necked trush (*Turdus albicollis*) sampled in the Brazilian Amazon (KU562808) and 99% identity to a *Plasmodium* sp. detected in a Magellanic penguin (*S. magellanicus*) captive at São Paulo Zoo, Southeastern Brazil (HM031936) (Fig. 3).

The Bayesian phylogenetical analysis based on a *cytB* gene 480 bp fragment and GTR + I + G evolution model positioned the obtained sequences in a group containing sequences of *Plasmodium* spp. detected in Passeriform birds sampled in the Brazilian Amazon (KU562808, JQ988724, KU562569, and KU562772) and *Plasmodium* sp., previously detected in a Magellanic penguin samples in the Zoo of São Paulo (HM031936) (Fig. 3). All the clades showed high statistical support values (posterior probability). All samples had a match to the lineage TURALB01 of *Plasmodium* sp. previously obtained from *T. albicollis* in Brazil (Fecchio et al. 2018).

Discussion

The diagnosis of avian malaria by *Plasmodium* sp. in *S. magellanicus* was obtained through the clinical, pathological and molecular methods. Avian malarial infections occur in a wide range of hosts (over 300 species of birds) and throughout the world, with the exception of the Antarctic continent (Valkiūnas 2005). Among penguins, at least 13 species, including *S. magellanicus*, are susceptible to *Plasmodium* infection (Vanstreels et al. 2016), and clinical disease usually occurs in penguins at rehabilitation centers and kept in captivity (Vanstreels et al. 2017). With respect to the susceptibility and specificity of hosts infected by *Plasmodium* spp., it is known that the same species of *Plasmodium* may affect many avian families and/or orders, with varying susceptibility and clinical signs intensity; yet, some species of *Plasmodium* appear to be relatively restricted to some avian species (Iezhova et al. 2005; Križanauskienė et al. 2006; Loiseau et al. 2010; Palinauskas et al. 2011). Penguins are, however, highly susceptible to the development of malarial infection that results in severe and lethal disease (Grilo et al. 2016), which is consistent with our findings showing that Magellanic penguins were extremely susceptible to the agent, whereas birds in adjacent enclosures were not affected.

In Brazil, many studies have been conducted to assess the diversity of haemosporidian protozoa infecting wild birds (both free-ranging and captive) and the influences of anthropized and preserved environments in the dynamic of infections (Bennett and Lopes 1980; Woodworth-Lynas et al. 1989; Ribeiro et al. 2005; Fecchio et al. 2007; Belo et al. 2009; Bueno et al. 2010; Lima et al. 2010; Sebaio et al. 2010; Fecchio et al. 2011, 2018). Thereby, previous reports in penguins in Brazil have included infections by *P. tejerai* in a rehabilitation center in Florianopolis (Silveira et al. 2013), *P. tejerai*, *P. cathemerium*, and *P. nucleophilum* in a rehabilitation center in Florianopolis (Vanstreels et al. 2014) and *P. cathemerium*, *P. nucleophilum*, and *P. unalis* in samples of penguins collected in five rehabilitation centers located in the coast region of Brazil (Vanstreels et al. 2015). In the present study, the *Plasmodium* species involved in this avian malaria infection was phylogenetically related to another sequence of *Plasmodium* sp. (HM031936) described previously in a São Paulo Zoo, Brazil, as *P. relictum* (Bueno et al. 2010). However, other authors indicated that this species corresponded, in fact, to a *Plasmodium* sp. lineage closely related to *P. lutzi* (Vanstreels et al. 2014).

Some features of the infection from the present study, such as the facts that all mortality occurred over a relatively short period (15 days) during summer and that the penguins were at the zoo for at least 1 year without any clinical signs, suggest that the infection occurred locally following admission to the zoo (Vanstreels et al. 2014; Vanstreels et al. 2015), and was unlikely to represent a recurrent asymptomatic infection (Palmer et al. 2013). Many wild birds present in the zoo may have acted as reservoirs for the agent, as proposed previously (Bueno et al. 2010). The *Plasmodium* spp. sequences obtained grouped with sequences from *Plasmodium* sp. detected in thrushes (*Turdus* spp.) in the Brazilian Amazon

Table 2 Tissue samples of Magellanic penguins (*Spheniscus magellanicus*) tested with nested PCR assays for the detection of *Plasmodium*/*Haemoproteus*

Penguin	Spleen	Brain	Skeletal muscle	Lungs	Liver
1	+	N/A	N/A	+	N/A
2	+	+	+	N/A	–
3	+	+	+	N/A	+

+: positive; −: negative; N/A: Not available

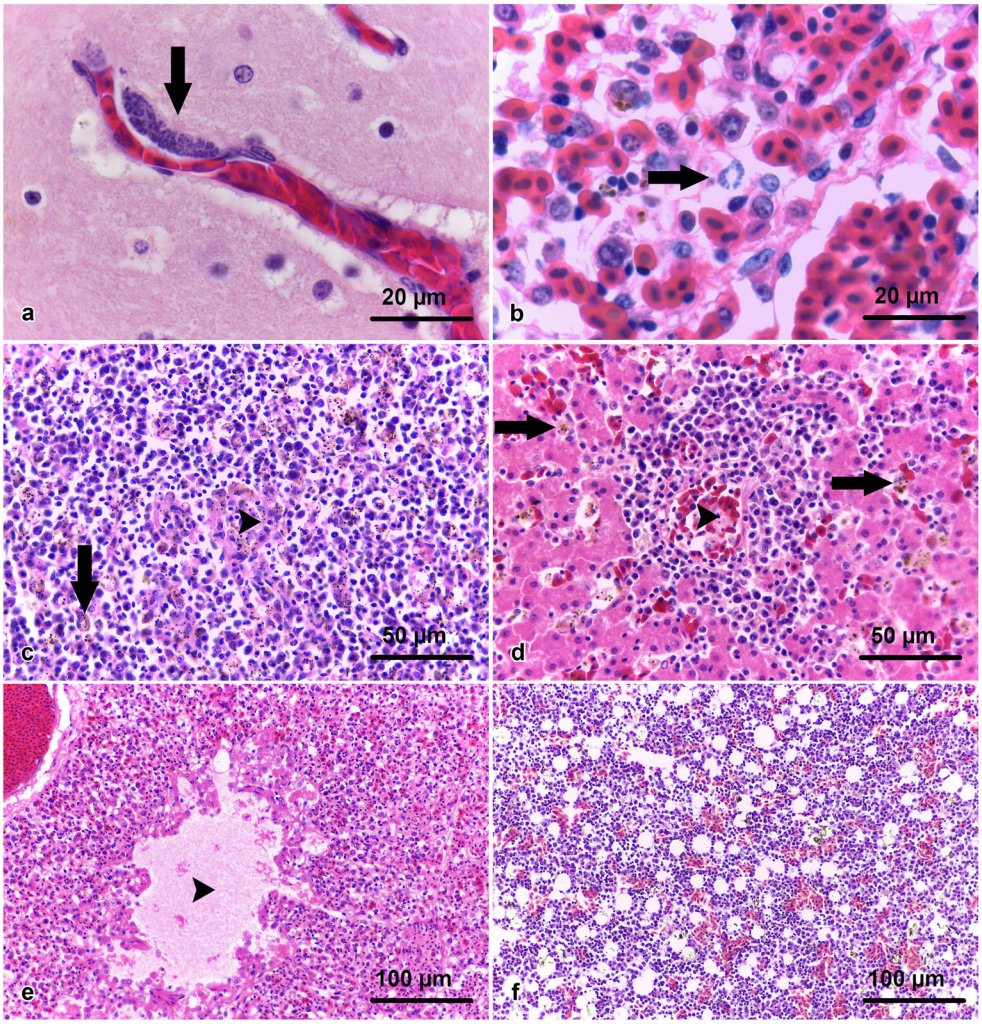

Fig. 2 Microscopical findings of *Plasmodium* sp. infection in captive Magellanic penguins (*Spheniscus magellanicus*) in South America. **a** Telencephalic cortex of penguin 2. Parasitary structures were observed in the cytoplasm of endothelial cells (arrow) and were composed of elongated exoerythrocytic meronts (15–25 μm × 8 μm) with multiple basophilic merozoites inside (1 μm in diameter). Hematoxylin and eosin (HE), × 1000. **b** Lungs of penguin 1. Parasitary structures were observed in the cytoplasm of endothelial cells, which were round and arranged in arcs (arrow), measuring 6–8 μm in diameter. HE, × 1000. **c** Spleen of penguin 3. Around blood vessels (arrow head) there was a severe inflammatory infiltrate composed by lymphocytes, plasma cells and macrophages, which frequently had hemosiderosis (arrow). HE, × 400. **d** Liver of penguin 2. A severe periportal to perivascular (arrow head) inflammatory infiltrate composed of lymphocytes, macrophages and occasional plasma cells. The sinusoids had also large amounts of macrophages with hemosiderosis (arrow). HE, × 400. **e** Lungs of penguin 1. Respiratory lobules adjacent to a parabronchi (arrow head) had a severe interstitial inflammatory infiltrate of macrophages. HE, × 200. **f** Bone marrow of penguin 2. There was a marked hyperplasia of the myeloid lineage. HE, × 200

(KU562808, JX029871, KU562569, and KU56772). Unfortunately, no other studies have examined the occurrence of blood parasites on free-living wild birds at the zoo, nor was there any information on the infection of other captive birds in adjacent enclosures; however, no mortality was noted. It is worth noting that *Turdus albicollis*, *T. amaurochalinus*, and *T. leucomelas* are widely present as free-living birds in the Rio Grande do Sul state (Bencke 2001), and, thus, *Plasmodium* sp. of lineage TURALB01 transmission may have occurred through the seasonal migration of these birds to the Southern Brazil. This hypothesis was validated in a previous study which showed that wild passerine birds, such as *T. merula* and *T. philomelos*, do not only act as a reservoir host for *Plasmodium*, but may also die due to avian malaria (Dinhopl et al. 2015). Avian malaria can be transmitted by mosquitos of the genus *Culex*, *Aedes*, *Culiseta*, *Anopheles*, *Mansonia*, and *Aedeomya* (Valkiūnas 2005). In a study performed at the São Paulo zoo, Brazil, *Culex*, *Aedes, Mansonia*, and *Anopheles* mosquito females were collected in the surrounding areas to the Magellanic penguins and submitted to the PCR for the detection of *Plasmodium* spp., resulting in the detection of only one positive mosquito (*Culex* spp) for *Plasmodium* sp., of which the sequence had 97–98% of identity to sequences of *Plasmodium* sp. obtained from penguins at the same facility (HM242420 e HM242419) (Bueno et al. 2010). The absence of control of mosquitoes in the enclosures where the penguins

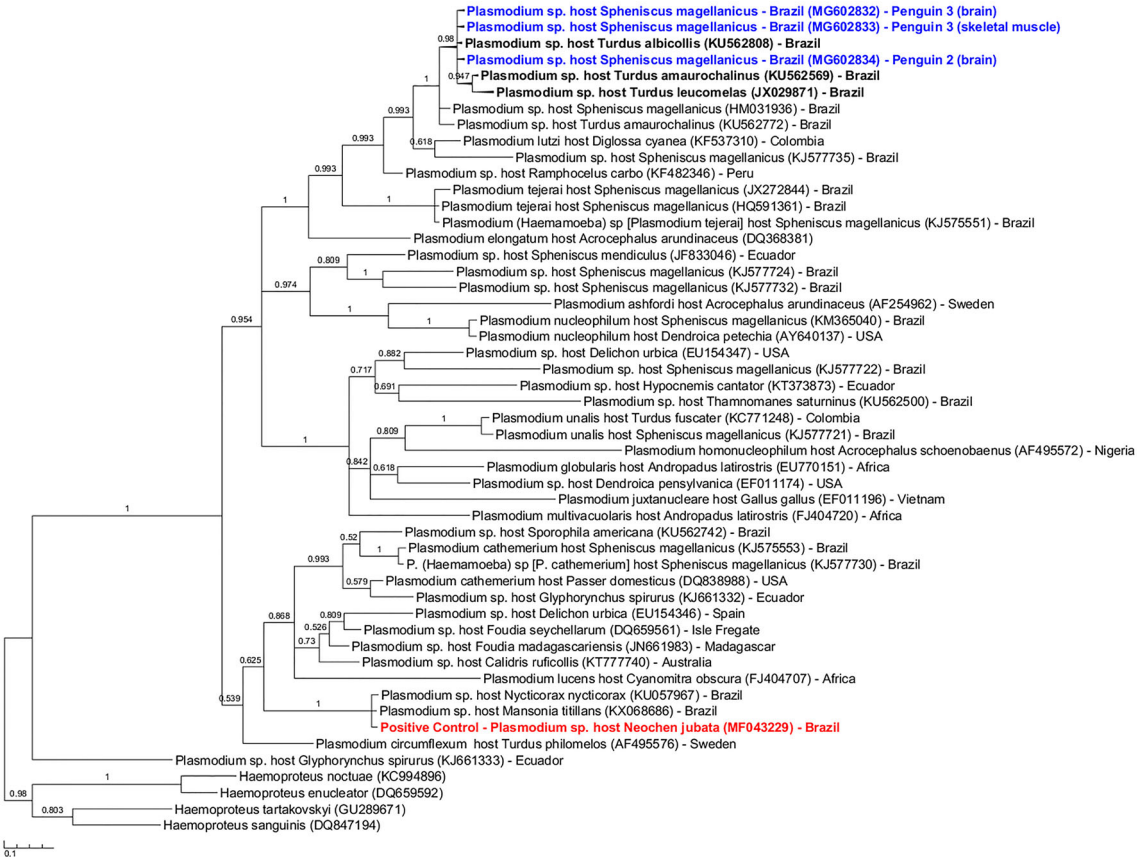

Fig. 3 Phylogenetical position of the sequences from *Plasmodium* sp. detected in Magellanic penguins (*Spheniscus magellanicus*) based on the Bayesian phylogenetical analysis of an alignment of 480 bp. The numbers in the branches of the tree indicate posterior probability values for the nodes (0–1). GenBank accession numbers are indicated aside to the sequences. The sequences from the present study are highlighted in blue, and the positive control is indicated

were housed may have been the most important predisposing factor to the infection in this study. The use of netting barriers in indoor and outdoor enclosure areas associated to quarantine and routine PCR assays for the detection of *Plasmodium* spp. may prevent the onset of avian malaria occurrence, and, consequently, these could prevent the high mortality caused by protozoa of the genus *Plasmodium* in penguins.

Clinical signs observed in avian malaria are only visible in the acute phase of the condition, varying from inactivity, weakness, dyspnea (Fix et al. 1988), and neurological signs (seizures) to sudden death (Bueno et al. 2010). In the present study, the penguins showed pale mucosa, dyspnea, and opisthotonos followed by death in a short clinical course, similarly to a peracute form of avian malaria in wild-caught *S. magellanicus* (Fix et al. 1988) and in two *S. magellanic* penguins kept in captivity (Silveira et al. 2013), which suggests that the penguins were not previously exposed to the agent and/or had low immunity to this, culminating with rapid death (Levin et al. 2009).

Morbidity and mortality related to avian malaria are remarkable in the acute phase of the disease, during which the intraerythrocytic multiplication of the agent occurs with resulting erythrocyte destruction and exoerythrocytic replication of the agent inside endothelial cells of many organs (Valkiūnas 2005). This may be related to the massive mortality observed in the present study, and it has been corroborated by the histological findings of exoerythrocytic meronts predominantly observed inside endothelial cells mainly in the brain, heart, and lungs, similarly to previous studies (Silveira et al. 2013; Vanstreels et al. 2014). The exoerythrocytic meronts located in the lung lead to clinical-pathological presentation of dyspnea and interstitial pneumonia of the condition, which has been previously associated to a severe pulmonary edema and respiratory failure in *S. magellanicus* (Fix et al. 1988) and *S. humboldti* (Bak et al. 1984), and probably led to the death of these animals. Although exoerythrocytic meronts located in the brain are less frequently described (Fix et al. 1988; Vanstreels et al. 2014), in some cases, death may result from the brain damage associated with exoerythrocytic merogony in capillaries irrigating the brain (Valkiūnas 2005). In the present study, endothelial cells of the brain were severely parasitized by *Plasmodium* sp., which probably caused a blood stasis with leptomeningeal blood vessels distension and neurological clinical signs observed at gross and clinical exams, respectively.

Predominant splenic and hepatic lesions, similarly to the ones observed, have been previously described as characteristics of avian malaria (Grim et al. 2003; Ko et al. 2008; Silveira et al. 2013; Vanstreels et al. 2014), and the enlargement of these organs observed grossly probably occurred due to extensive inflammatory infiltrate that was present microscopically, which was previously described as a reticuloendothelial hyperplasia in response to the *Plasmodium* infection (Bak et al. 1984). However, the presence of exoerythrocytic meronts in these lesions is rare (Silveira et al. 2013; Vanstreels et al. 2014), with a preponderance of the inflammatory response to the hemolytic disease with erythrophagocytosis and hemosiderosis concomitantly. Besides that, severe perivascular mononuclear to histiocytic splenitis and hepatitis were also observed, which have been previously described as consequences of the vasculitis associated to the proliferation of the agent in endothelial cells (Ko et al. 2008; Vanstreels et al. 2015); however, vascular inflammatory reactions were absent.

Avian malaria infection by *Plasmodium* sp. may result in a peracute clinical course disease with high mortality in Magellanic penguins. At necropsy, marked splenomegaly and hepatomegaly are characterized. Microscopically, numerous exoerythrocytic meronts are observed inside endothelial cells of many organs. The molecular characterization of the agent involved is essential to obtain a differential diagnosis of the condition and to classify the lineage of *Plasmodium* sp. involved. Preventive measures aiming control of mosquitoes in rehabilitation centers and zoos are important to avoid the infection.

Source of funding This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Compliance with ethical standards The authors declared that there was explicit owner informed consent for inclusion of animals in this study. All co-authors approved the manuscript and its submission to the journal.

Conflict of interest The authors declare that they have no conflicts of interest.

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