

## Avian Malaria (*Plasmodium* spp.) in Captive Magellanic Penguins (*Spheniscus magellanicus*) from Northern Argentina, 2010

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**ABSTRACT:** We report two cases of lethal avian malaria in Magellanic Penguins (*Spheniscus magellanicus*) captive at San Clemente del Tuyú, Argentina, approximately 560 km north of Argentinean breeding colonies of Magellanic Penguins. Blood smears revealed both penguins were concurrently infected by *Plasmodium* (*Haemamoeba*) *tejerai*, *Plasmodium* (*Huffia*) sp., and *Plasmodium* (*Novyella*) sp.

Avian malaria is a disease caused by mosquito-transmitted protozoans of the genus *Plasmodium* (Valkiūnas 2005). Penguins are known to be highly susceptible to these pathogens, and outbreaks in zoos often lead to high morbidity and mortality (Fix et al. 1988; Vanstreels et al. 2014). Magellanic Penguins (*Spheniscus magellanicus*) breed along the coast of central and southern Argentina, Chile, and the Falkland Islands, and seasonally migrate to northern Argentina, Uruguay, and Brazil (Stokes et al. 2014). *Plasmodium* infection has never been reported in wild Magellanic Penguins; however, it has been documented in zoos in Brazil (Bueno et al. 2010), the US (Fix et al. 1988), and South Korea (Ko et al. 2008) and at rehabilitation centers in northern Chile (Carvajal and Alvarado 2009) and Brazil (Silveira et al. 2013; Vanstreels et al. 2014, 2015).

In March 2010, two adult Magellanic Penguins that were permanently held at Oceanarium Mundo Marino (San Clemente del Tuyú, Argentina; 36°20'17"S, 56°45'14"W) died suddenly. In both cases, necropsy revealed liver and spleen enlargement and hydropericardium, and Giemsa-stained liver impression smears presented tissue meronts compatible with *Plasmodium*. Blood smears

were obtained approximately 1 wk prior to death. Blood parasites were morphologically characterized (Valkiūnas 2005), revealing that at least three species of *Plasmodium* were present in both cases (Fig. 1): *Plasmodium* (*Haemamoeba*) *tejerai*, *Plasmodium* (*Huffia*) sp., and *Plasmodium* (*Novyella*) sp.

*Plasmodium tejerai* was identified based on the presence of (a) ring-shaped trophozoites in mature erythrocytes, with a large central vacuole, often displacing the host cell nucleus; (b) roundish or oval meronts with plentiful cytoplasm, larger than the host cell nucleus and often displacing it, containing 12 to 15 round, oval, or irregular merozoites that were distributed randomly or in rosettes; and (c) round, oval, or irregular gametocytes, larger than host cell nucleus and often displacing it, often presenting lobular or irregular pointed projections.

An unidentified lineage of the subgenus *Huffia* was recognized based on the presence of (a) round or oval trophozoites in immature erythrocytic lineage cells and (b) irregularly shaped meronts with abundant cytoplasm in both mature erythrocytes and immature erythrocytic lineage cells, often larger than host cell nucleus and often displacing it, containing 6–16 round merozoites that were randomly distributed.

An unidentified lineage of the subgenus *Novyella* was recognized based on the presence of (a) ring-shaped or irregular trophozoites in mature erythrocytes, which did not displace the host cell nucleus, and (b) compact or irregularly shaped meronts with scanty cytoplasm, smaller than host cell

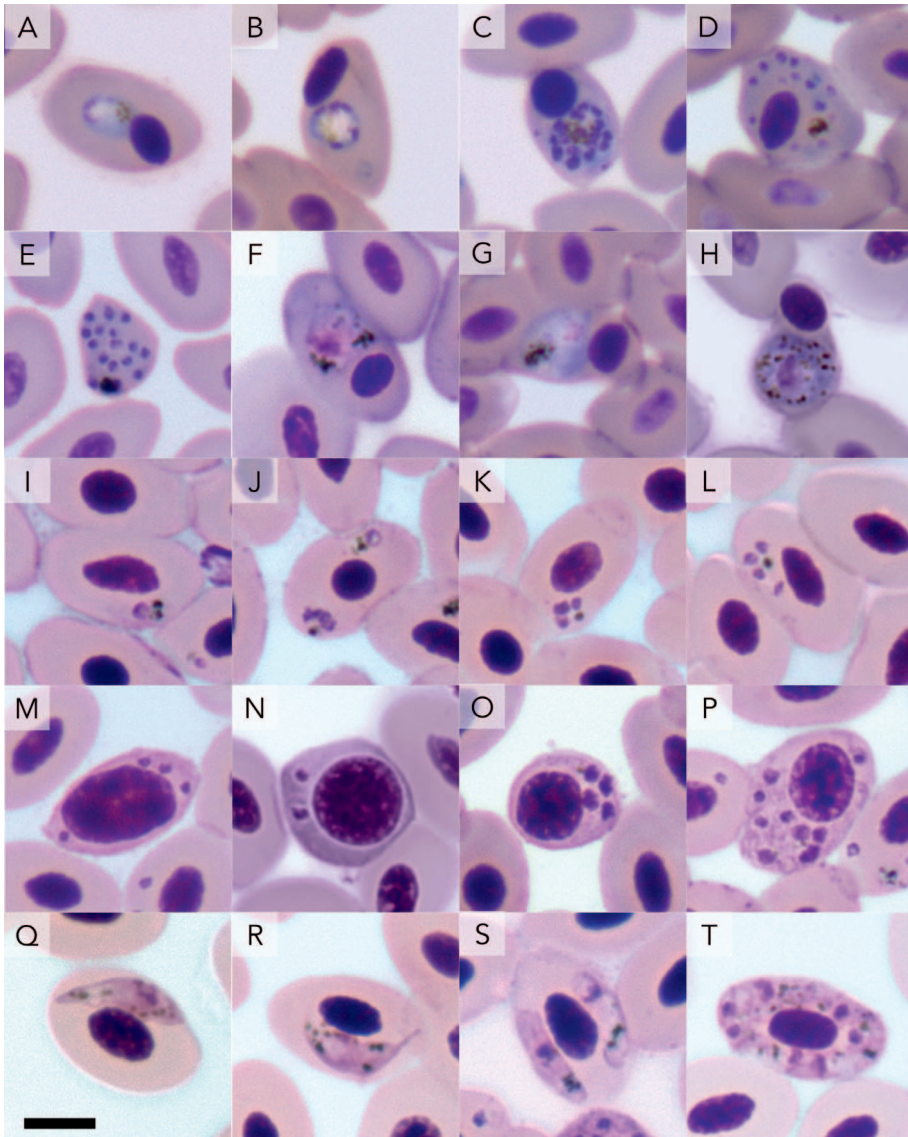


FIGURE 1. *Plasmodium* spp. in Giemsa-stained blood smears of a Magellanic Penguin (*Spheniscus magellanicus*; Case A), from northern Argentina, 2010. Photomicrographs: (A, B) *Plasmodium tejerai* trophozoites, (C, D, E) *P. tejerai* meronts, (F, G, H) *P. tejerai* gametocytes, (I) *Plasmodium* sp. trophozoite, (J) co-infection by *Plasmodium* sp. trophozoite and *Plasmodium (Novyella)* sp. meront, (K, L) *P. (Novyella)* sp. meronts, (M, N) *Plasmodium (Huffia)* sp. trophozoites, (O, P) *P. (Huffia)* sp. meronts, (Q, R) *Plasmodium* sp. elongated gametocytes, (S, T) co-infection by *Plasmodium* sp. elongated gametocytes. Bar=5  $\mu$ m.

nucleus and not displacing it, containing 4–6 round or oval merozoites that were frequently organized in rosettes.

Elongated gametocytes were also observed, slightly longer than and approximately as wide as the host cell nucleus, at the lateral or subpolar position, and displacing the host cell

nucleus laterally only slightly or not at all; these were considered compatible with both *Plasmodium (Huffia)* sp. and *Plasmodium (Novyella)* sp.

Parasitemia was quantified manually from 1,000 erythrocytes. Cases A and B had, respectively, 0.33 and 0.03 parasites/erythro-



FIGURE 2. Geographic distribution of *Plasmodium tejerai* records. The breeding (dark shading) and wintering distribution (light shading) of Magellanic Penguins (*Spheniscus magellanicus*) is also represented (Birdlife International 2012).

cyte, with 7.62% and 0.34% of erythrocytes infected by two or three parasites. The relative parasitemia of each parasite species could not be reliably established.

*Plasmodium tejerai* was first described infecting turkeys in Trujillo, Miranda province, Venezuela (Gabaldón and Ulloa 1977). Subsequently it was identified in Magellanic Penguins at a rehabilitation center in Florianópolis, Brazil in 2009 (Silveira et al. 2013; Vanstreels et al. 2014, 2015). In this study we found *P. tejerai* in San Clemente del Tuyú, more than 1,200 km away from Florianópolis and 5,200 km from Trujillo (Fig. 2), demonstrating that *P. tejerai* has a broad Neotropical distribution. It is puzzling that recent studies have identified *P. tejerai* only in Magellanic Penguins, whereas no studies have identified the infection in other avian hosts since its

original description. Three hypotheses may explain this: (a) There is a scarcity of studies examining the blood parasites of nonpenguin birds in southernmost South America; (b) *P. tejerai* tends to produce low parasitemia infections that are difficult to detect in other avian hosts, whereas Magellanic Penguins develop high parasitemia that can be readily detected; or (c) the lack of gene sequences of this parasite in public databases until 2013 has led this parasite to be misidentified in other studies.

*Plasmodium (Huffia) elongatum* has been frequently documented infecting penguins in North America and South America (Beier and Stoskopf 1980; Vanstreels et al. 2015). Most morphologic characteristics of the *P. (Huffia)* sp. observed in this study were consistent with *P. elongatum*; however, the merozoites within the erythrocytic meronts of the *P. (Huffia)* sp. were considerably larger and the erythrocytic meronts often occupied the entire the host cell cytoplasm (which is not usual in *P. elongatum*). It is therefore not clear whether this *P. (Huffia)* sp. is a different species, perhaps novel, or if these cases corresponded to *P. elongatum* with morphologic aberrations related to the high parasitemia and multiple infection.

Two species of the subgenus *Novyella* (*P. nucleophilum* and *P. unalis*) have been documented infecting Magellanic Penguins (Vanstreels et al. 2015). The high parasitemia and multiple infection precluded the conclusive identification of the *P. (Novyella)* sp. observed in this study. Nonetheless, several morphologic characteristics of the erythrocytic meronts suggest *P. vaughani* as the most probable morphospecies: presence of a refringent globule, amoeboid shape of erythrocytic meronts, infection of both mature and immature erythrocytes, number of merozoites not higher than six, polar or subpolar position, and not in direct contact with the nuclear membrane.

This is the southernmost record of avian malaria in penguins on the Atlantic coast, approximately 560 km north of the closest Magellanic Penguin breeding colony (Complejo Islote Lobos, 41°25'S). Unlike in San

Clemente del Tuyú, however, mosquitoes are uncommon in the Argentinean Patagonia due to its arid climate (Mitchell and Darsie 1985). The geographic distribution of avian malarial parasites has been shifting due to climate change (Garamszegi 2011), the breeding distribution of Magellanic Penguins is shifting northwards (Gómez-Laich et al. 2015), and human occupation of the Patagonian coast could result in mosquito-friendly microhabitats (freshwater availability, wind protection, etc.). It is therefore possible that an overlap could arise between the distribution of avian malarial parasites and Magellanic Penguins in northern Argentina.

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