










***Mycoplasma* spp. in Psittaciformes kept as pets in the southwestern Amazon of Brazil¹**

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ABSTRACT. Nascimento BKF, Medeiros LS, Machado LS, Carvalho LA, Cordeiro ALL, Satrapa RA, Ribeiro VMF. ***Mycoplasma* spp. in Psittaciformes kept as pets in the southwestern Amazon of Brazil.** *Pesquisa Veterinária Brasileira* 45:e07557, 2025. Bloco De Medicina Veterinária, Universidade Federal do Acre, Rodovia BR-364 Km 4, Distrito Industrial, Rio Branco, AC 69920-900, Brazil. E-mail: vania.rib@uol.com.br

Mycoplasmosis, caused by *Mycoplasma* spp., is a disease of great importance in birds, especially in the poultry industry. They are considered emerging bacteria that cause subclinical and clinical diseases in various birds and should not be ruled out as responsible for infections in Psittaciformes. Their development may go unnoticed, given their slow evolution, which ends up generating difficulties in the diagnosis and treatment of these animals. Thus, this research aims to detect the presence of *Mycoplasma* spp. in Psittaciformes kept as pets in the southwestern Amazon of Brazil. The animals in the study were kept as pets and were selected based on convenience criteria. Biological material was collected using swabs from the oral and cloacal cavities of 100 birds. Subsequently, PCR was performed in all samples to identify *Mycoplasma* spp. The results demonstrated the presence of DNA from *Mycoplasma* spp. in 7% (7/100) of the biological samples of the investigated Psittaciformes.

INDEX TERMS: *Mycoplasma* spp., wild birds, avian mycoplasmosis, PCR.

RESUMO.- [*Mycoplasma* spp. em Psittaciformes mantidos como pet na Amazônia Sul Ocidental do Brasil.]

A micoplasmose aviária, causada por *Mycoplasma* spp. é uma enfermidade de grande importância nas aves, principalmente na indústria avícola. São consideradas bactérias emergentes, que causam doenças subclínicas e aparentes em diversas aves, e não devem ser descartadas como responsáveis por infecções em Psittaciformes. O desenvolvimento pode passar despercebido, dado o seu caráter de evolução lenta, o que acaba por gerar uma dificuldade no diagnóstico e no tratamento desses animais. Dito isto, esta pesquisa tem como objetivo

detectar a presença de *Mycoplasma* spp. em Psittaciformes mantidos como pet na Amazônia Sul Ocidental do Brasil. Os animais do estudo eram criados como pets e a seleção se deu por critério de conveniência. Foram realizadas coletas de material biológico com swabs na cavidade oral e cloacal em 100 aves. Posteriormente, foi processado o PCR para identificação de *Mycoplasma* spp. em todas as amostras. Os resultados demonstraram a presença de DNA da bactéria *Mycoplasma* spp. em 7% (7/100) das amostras biológicas dos Psittaciformes investigados.

TERMOS DE INDEXAÇÃO: *Mycoplasma* spp., aves silvestres, micoplasmose aviária, PCR.

INTRODUCTION

The order Psittaciformes comprises three families: Loridae (lories); Cacatuidae (cockatoos); and Psittacidae (parrots, macaws, parakeets, conures, and jandays) (Sick 1997). It consists of approximately 78 genera and 332 species, with 148 species found in the New World and 184 in the Old World. South America is particularly rich in psittacine diversity, harboring approximately 100 species, with Brazil alone accounting for 80 – making it the most species-rich

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country for psittacines and earning it the nickname “Land of parrots” (Collar 1997, Sick 1997). Notably, 16 of these 80 species are listed as threatened with extinction in the Red Book of Brazilian Fauna Threatened with Extinction (Machado et al. 2008).

According to Destro et al. (2012), birds represent approximately 81% of the animals rescued by the “Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis” (National Institute of the Environment and Renewable Natural Resources – IBAMA) from 2002 to 2009. The “Centro de Triagem de Animais Silvestres” (Wildlife Rehabilitation Center – CETAS) of the State of Acre received a total of 2,320 wild animals from 2010 to 2014. Of this total, 1,097 (47.2%) were birds. Among them, *Amazona ochrocephala* (yellow-crowned parrot), a psittacine species, accounted for 112 individuals (10.2%), making it the second most rescued species after *Sporophila angolensis* (curió) (Nascimento et al. 2016).

Over the decades, wild animals have increasingly been kept as companion animals, leading to closer contact between wild animals and humans. As a result, the risk of zoonotic disease transmission has increased drastically. Birds, particularly psittacines, can be reservoirs or carriers of zoonoses, such as psittacosis, salmonellosis, and mycoplasmosis, all of which represent serious public health concerns (Torres et al. 2016).

Mycoplasmosis, caused by *Mycoplasma* spp., is a significant disease in birds, particularly within the poultry industry. The most economically impactful species of bacteria is *Mycoplasma gallisepticum*, followed by *Mycoplasma synoviae*, *Mycoplasma iowae* and *Mycoplasma meleagridis* (Buim et al. 2009). These bacteria are considered emerging pathogens causing subclinical and clinical diseases in various bird species, including psittacines (Nascimento et al. 1982, Yoder 1991, Kleven 2003). Furthermore, they have already been detected in *Amazona aestiva* (blue-fronted parrot), where they are responsible for 20% mortality (Bozeman et al. 1984).

Mycoplasma are the smallest known bacteria, comparable in size to large viruses (~300 nm). They have a diverse distribution and are reported in plants, fish, reptiles, birds, mammals, and humans. In animals, they can cause respiratory, joint, and urogenital disorders (Rivera-Tapia et al. 2001, Nascimento & Pereira 2009). Their transmission can occur horizontally through various means, including mating, aerosols, water, food, fomites, and direct contact with infected birds (Berchieri Junior 2000).

The development of these diseases in psittacines may go unnoticed, given their slow progression, making diagnosis and treatment challenging (Tully & Harrison 1994). Timely diagnosis and treatment of these diseases are crucial because releasing rescued animals may facilitate the dissemination of these pathogens to wild animals. Moreover, disease control in captive animals is essential to minimize the risk of infection in humans (Carvalho 2012).

Although psittacines are the most commonly rescued animals in inspections conducted by environmental agencies in the State of Acre, located in the Amazon, there are only a few records of *Mycoplasma* spp. in these birds. Therefore, the objective of this study was to detect the presence of *Mycoplasma* spp. in pet psittacines in the southwestern Amazon region of Brazil.

MATERIALS AND METHODS

Ethical approval. This study was authorized by the Ethics Committee on Animal Use of the “Universidade Federal do Acre” (UFAC) under process number 23107.019896/2017-64 and protocol number 40/2017.

Animals, location and anamnesis. The study involved Psittaciformes kept as pets in both urban and rural areas of Rio Branco/AC, in the southwestern Amazon region of Brazil.

The animals were selected based on convenience sampling, depending on the owner’s authorization and availability to receive the research team for the collection of biological materials. A questionnaire was administered to the owners to collect information about sanitary management, level of interaction with the owner, type of confinement, possible contact with other animals, feeding habits, and medical history.

A total of 100 Psittaciformes of undefined age were included in this study. Sample collection was conducted from May to September 2019, employing physical restraint techniques to prevent thoracic compression and ensure normal respiratory movement, thereby avoiding asphyxiation, in accordance with previously established guidelines (Benez 2004).

Collection of biological materials for *Mycoplasma* spp.

Oropharyngeal and cloacal samples were collected from all animals using sterile swabs, resulting in a total of 200 samples (Fig. 1), followed by accurate labeling with the corresponding animal’s record number. Samples were stored at -20 °C in the Molecular Epidemiology Laboratory of the “Departamento de Saúde Coletiva Veterinária e Saúde Pública” (Department of Veterinary Collective and Public Health) at “Universidade Federal Fluminense” (UFF) until further processing.

Laboratory processing for detection of *Mycoplasma* spp.

Polymerase chain reaction (PCR) was initially performed to identify *Mycoplasma* spp. using a technique adapted from Van Kuppeveld

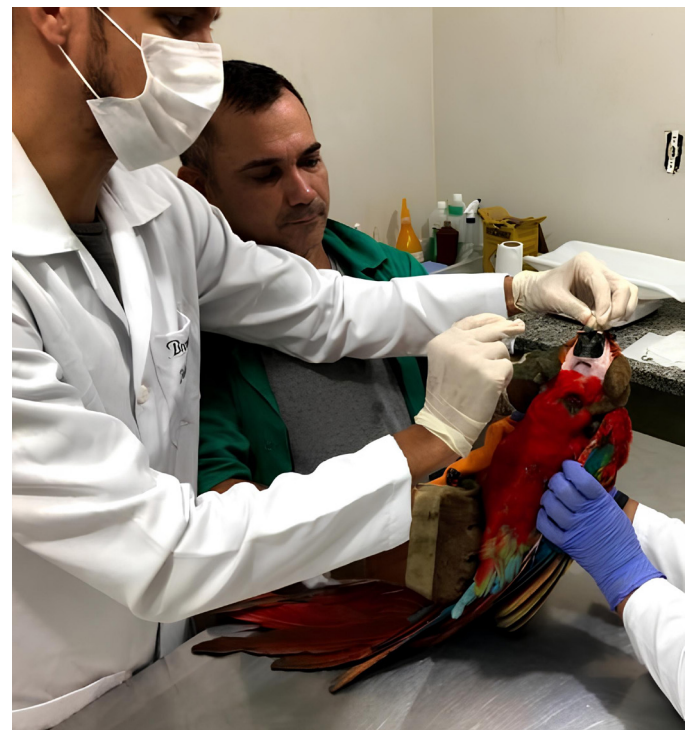


Fig. 1. Collection of oropharyngeal mucosa samples using swabs.

et al. (1994). Nucleic acids were isolated using sodium dodecyl sulfate and proteinase K, extracted using phenol and chloroform, and subsequently precipitated. The products were resuspended with 50 µl of distilled water. Amplification was performed with a mixture of ultrapure water, 10× buffer (1×), 50 mM MgCl₂ (2.0 mM), 10 mM dNTP (0.2 mM), 10 µM GPO3 primer (0.2 mM), 10 µM MGS0 primer (0.2 mM), and 5 U/µL Taq (1U). The primers used were GPO-3 (5'-GGG AGC AAA CAG GAT TAG ATA CCC T-3') and MGS0 (5'-TGC ACC ATC TGT CAC TCT GTT AAC CTC-3'), which amplify a 280-bp fragment. For the cycles, the first stage consisted of 1 cycle at 94 °C for 5 min, followed by a second stage of 40 cycles at 94 °C for 1 min, 40 cycles at 55 °C for 1 min and 40 cycles at 72 °C for 2 min. In the third stage, one cycle was performed at 72 °C for 10 min, followed by cooling to 4 °C. Subsequently, the positive samples were subjected to a new PCR to verify the presence of *M. gallisepticum* and/or *M. synoviae*.

In PCR for *M. gallisepticum*, the technique adapted from Nascimento et al. (1991) was employed with ultrapure water, 10× buffer (1×), 50 mM MgCl₂ (2.0 mM), 10 mM dNTP (0.2 mM), 10 pmol Primer B1 (0.5 µM), 10 pmol Primer B2 (0.5 µM), 5 U/µL Taq (1U). The primers used were B1 (5'-GGA TCC CAT CTC GAC CAC GAG AAA A-3') and B2 (5'-CTT TTC AAT CAG TGA GTA ACT GAT GA-3'), which amplify a 732-bp product. The thermal cycling protocol included an initial stage of 1 cycle at 95 °C for 5 min, followed by 30 cycles at 95 °C for 1 min, 30 cycles at 55 °C for 2 min, 30 cycles at 72 °C for 1 min, and a final stage of 1 cycle at 72 °C for 5 min, with cooling at 4 °C.

In PCR for *M. synoviae*, the technique was adapted from Lauerman et al. (1995), using ultrapure water, 10× buffer (1×), 50 mM MgCl₂ (2.0 mM), 10 mM dNTP (0.2 mM), 10 pmol/µL MSf primer (0.2 mM), 10 pmol/µL MSr primer (0.2 mM) and 5 U/µL Taq (1.25 U). The primers used were MS-f (5'-GAG AAG CAA AAT AGT GAT ATC A-3') and MS-r (5'-CAG TCG TCT CCG AAG TTA ACA A-3'), which amplify a 486-bp product. For the cycles, a first stage of 1 cycle at 94 °C for 1 min was performed, followed by a second stage of 40 cycles at 94 °C for 30 s, 40 cycles at 55 °C for 30 s, 40 cycles at 72 °C for 1 min and a last stage of 1 cycle at 72 °C for 5 min, followed by cooling to 4 °C.

The PCR products were analyzed by electrophoresis on 1.5% agarose gels stained with ethidium bromide (0.5 mg/ml) and visualized under ultraviolet light.

RESULTS

Species of the collected samples, the total number of samples per species, and the number of positive results for *Mycoplasma* spp. are summarized in Table 1. Among the samples collected from swabs of the oral mucosa of Psittaciformes, *Mycoplasma* spp. was detected in seven out of 100 (7%) birds. No swabs collected from the cloacal mucosa tested positive for *Mycoplasma* spp. None of the samples tested positive for *M. gallisepticum* or *M. synoviae*. *Amazona ochrocephala* had the highest number of positive samples (3/7, 42.86%), followed by *Nymphicus hollandicus* (cockatiel) and *Pionus menstruus* (blue-headed parrot), with both species accounting for 28.57% (2/7) each. Among the species that tested positive for *Mycoplasma* spp., *P. menstruus* exhibited dyspnea, a clinical sign consistent with mycoplasmosis, whereas the other species did not present any apparent clinical signs.

A significant finding of the present study was the proximity of owners to their birds, which was characterized as close (owner-bird interaction throughout the day), moderate (interaction during part of the day), and minimal (limited to cleaning the enclosure and refilling water and food). It was evaluated that of the 100 birds, 24% had very close contact, 56% had moderate contact, and 20% had negligible contact.

Of the 100 Psittaciformes evaluated, free-ranging Psittaciformes comprised 20%, 33% lived in semi-confinement (free-range for a few hours a day), and 47% were permanently confined. Among the seven birds that tested positive for *Mycoplasma* spp., three lived in a free-range system, two in a semi-confinement system, and two in a permanent confinement system (Table 2). Of the 100 individuals collected, 19% had contact with other animals such as cats, dogs, and other birds (domestic or free-ranging). Among the seven animals that tested positive for *Mycoplasma* spp., four had routine contact with dogs and free-ranging birds, one had contact with dogs, and two did not interact with any other animals, according to the owners. Moreover, owners reported that *Columba livia* and birds of the genus *Columbina*, the free-ranging birds, were interacting most frequently with the captive Psittaciformes.

Table 1. Scientific and common names, amount of animals sampled, and species with positive PCR results for *Mycoplasma* spp. in Psittaciformes kept as pets in the southwestern Amazon of Brazil

Scientific name	Common name	Amount	Positive for <i>Mycoplasma</i> spp.
<i>Amazona ochrocephala</i>	Yellow-crowned parrot	30	3
<i>Nymphicus hollandicus</i>	Cockatiel	17	2
<i>Melopsittacus undulatus</i>	Budgerigar	13	0
<i>Aratinga aurea</i>	Peach-fronted parakeet	11	0
<i>Amazona aestiva</i>	Blue-fronted parrot	6	0
<i>Pionus menstruus</i>	Blue-headed parrot	6	2
<i>Ara macao</i>	Scarlet macaw	3	0
<i>Aratinga weddellii</i>	Dusky-headed parakeet	3	0
<i>Primolius maracana</i>	Blue-winged macaw	3	0
<i>Brotogeris sanctithomae</i>	Tui parakeet	2	0
<i>Agapornis</i>	Love birds	2	0
<i>Ara severus</i>	Chestnut-fronted macaw	2	0
<i>Psittacara leucophthalmus</i>	White-eyed conure	1	0
<i>Amazona festiva</i>	Festive parrot	1	0
TOTAL	-	100	7

DISCUSSION

In the present study, 7% (7/100) of the birds tested positive for *Mycoplasma* spp. in the oral swab samples. The higher sensitivity of the test using oral swabs compared to that using cloacal swabs may be related to the initial sites of bacterial contamination, which have a predilection for the upper respiratory tract (Gomes et al. 2010). *Amazona ochrocephala* and *Nymphicus hollandicus* were most commonly found in homes in both urban and rural areas of Rio Branco, Acre, totaling 30 and 17 individuals, respectively. A greater number of samples of these species were observed in the PCR tests, with three yellow-crowned parrots and two cockatiels positive for *Mycoplasma* spp. The remaining two positive individuals were *Pionus menstruus*, which comprised six of the 100 individuals collected. The native species *Amazona aestiva* and *Ara ararauna* (blue-and-yellow macaw) are the most commercially desired species and are found in large numbers in breeding facilities, zoos, and wildlife rescue centers (Pinho & Nogueira 2000, Nascimento et al. 2010). Therefore, these two species are frequently reported in studies on the occurrence of *Mycoplasma* spp. in psittacines conducted throughout Brazil (Gomes et al. 2010, Silva et al. 2016, Carvalho et al. 2017, Magalhães et al. 2020). However, in the present study, only six individuals of *A. aestiva* were collected, none of which tested positive for *Mycoplasma* spp. Furthermore, no specimens of the *A. ararauna* were observed during home visits in either urban or rural areas of the southwestern region of the Brazilian Amazon.

Gomes et al. (2010) collected cloacal swab samples from 77 psittacines (captive and free-ranging) in the southeastern region of Brazil and reported a rate of 53.5% positivity for *Mycoplasma gallisepticum* after PCR testing. In a study by Carvalho et al. (2017) conducted in the central region of Brazil, 34.15% (14/41) of oral swab samples from captive psittacines were positive for *M. gallisepticum*, and 7.32% (3/41) were positive for *M. synoviae*. Moreover, samples of *A. ochrocephala* were collected with an infection rate of 25% (1/4). Silva et al. (2016) analyzed 85 psittacines kept in captivity in northeastern Brazil and reported *Mycoplasma* spp. in 16.47% of the birds. Magalhães et al. (2020) investigated the occurrence of *Mycoplasma* spp. in 45 Psittaciformes from the Rio de Janeiro City Zoo and detected its presence in 53.33% of the individuals. Notably, the present study reported a lower occurrence rate (7%) of *Mycoplasma* spp. compared to similar studies in the literature. Assessing the methodological differences, it is evident that the present study, unlike others in which the birds originated mainly from zoos and breeding facilities (Gomes et al. 2010, Silva et al. 2016, Carvalho et

al. 2017, Magalhães et al. 2020), analyzed psittacine birds raised as pets in homes in rural and urban areas, which are characterized by less contact with other birds, whether free-living or kept in captivity. This difference in housing may have contributed to a relatively low occurrence of the agent, as it is known that keeping a large number of birds in enclosures and cages promotes greater contact between animals, predisposing the spread of the pathogen through aerosols (Fischer et al. 1997). Gomes et al. (2010) suggested that factors such as high density, poor hygiene, stress, and contact with other birds might contribute to the high prevalence of this pathogen in psittacines. This transmission occurs through the contact of contaminated droplets with the host's conjunctival and upper respiratory tract mucous membranes (Nascimento & Pereira 2009, Stipkovits & Szathmary 2012).

With the presence and subsequent proliferation of these preferred entry routes, this agent is capable of promoting respiratory and reproductive clinical signs in the affected animals (Carnaccini et al. 2016). In the present study, only one individual exhibited symptoms (dyspnea) indicating mycoplasmosis. Moreover, studies investigating this pathogen frequently report birds that are positive for *Mycoplasma* spp. remain asymptomatic, regardless of the contamination rates (Silva et al. 2016, Carvalho et al. 2017, Magalhães et al. 2020, Lugarini et al. 2021). Like other bacteria of the Mollicutes class, *Mycoplasma* spp. act in symbiosis with the infected animal, where causing severe damage to the host may prove detrimental to the pathogen itself, usually resulting in chronic or subclinical diseases (Razin et al. 1998). Furthermore, a higher frequency of low pathogenic strains in certain samples may contribute to the lower incidence of observable clinical signs. It is well established that *Mycoplasma* strains vary widely in their pathogenic potential (Lecis et al. 2010).

In the present study, four of the seven birds that tested positive for *Mycoplasma* spp. through oral swab samples, were observed in close proximity to free-living birds. The presence of free-living birds, such as the Columbiforms, observed during sample collection may contribute to the number of positive cases, as these species are considered potential dispersers of this bacterium and exhibit a high degree of adaptation to *M. gallisepticum* (Gharaibeh & Hailat 2011). Furthermore, there are reports of the occurrence of *Mycoplasma* spp. reaching 22.2% in free-living Columbiform birds in northeastern Brazil (Lugarini et al. 2021). The risk of infection by *Mycoplasma* spp. increases when captive birds are in close proximity to free-ranging urban birds (O'Connor et al. 1999, Nolan et al. 2000).

Although rare, the zoonotic potential of mycoplasmas is documented in the literature, particularly in immunosuppressed

Table 2. Scientific names and identification, housing systems and contact with other animals related to Psittaciformes that tested positive in the polymerase chain reaction (PCR) test for detection of *Mycoplasma* spp. in oral swab samples

Scientific name	Housing system	Contact with other animals
<i>Amazona ochrocephala</i> – A3	Free	Free-living birds and dogs
<i>A. ochrocephala</i> – A9	Semi-confinement	Free-living birds and dogs
<i>A. ochrocephala</i> – A21	Confined	None
<i>Nymphicus hollandicus</i> – A5	Confined	None
<i>N. hollandicus</i> – A10	Semi-confinement	Dogs
<i>Pionus menstruus</i> – A1	Free	Free-living birds and dogs
<i>P. menstruus</i> – A11	Free	Free-living birds and dogs

patients (Heller et al. 2015), in cases of occupational infections among biologists and veterinarians working with seals (White & Jewer 2009), and in pigs (Yuan et al. 2009). In the present study, close contact between psittaciform birds and their owners was observed in 24% of the analyzed animals. In addition, the vast majority of infected animals were asymptomatic. Animals without clinical signs can harbor these bacteria and become silent disseminators because their clinical signs are only expressed when the carrier is immunosuppressed (Razin et al. 1998). Furthermore, birds that tested negative for *Mycoplasma* spp. have zoonotic potential for several other pathogens, such as *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Acinetobacter* spp., *Streptococcus*, *Staphylococcus*, and *Lactobacillus*, according to Dorrestein et al. (1985) and Corrêa et al. (2013).

CONCLUSION

The presence of DNA from *Mycoplasma* spp. in 7% of the biological samples of the studied Psittaciformes appears to be the first report on the identification of bacteria in Psittaciformes kept as pets in the southwestern Amazon. All the analyzed birds were kept as pets, and 24% were in close proximity to their owners. Reports of interspecific transmission in the scientific literature indicate zoonotic potential, which should be further investigated.

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Credit author statement.- Breno KFN and Vânia MFR: conceptualization; Breno KFN, Luciana SM, Leandro SM and Lucas AC: methodology; Breno KFN, Luciana SM, Andrey LLC and Rafael AS: formal analysis; Breno KFN, Luciana SM, Andrey LLC and Rafael AS: investigation; Rafael AS, Luciana SM and Vânia MFR: data curation; Breno KFN and Andrey LLC: writing – original draft; Andrey LLC, Rafael AS and Vânia MFR: writing – review and editing; Rafael AS and Vânia MFR: supervision. All authors have read and agreed to the published version of the manuscript.

Data availability statement.- The raw data supporting the conclusions of this article will be made available by the authors on request.

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