ORIGINAL ARTICLE

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Screening of *Salmonella* spp. and *Chlamydophila psittaci* in parrots domiciled in Rio Branco, Acre, Brazil

Salmonella *spp. e* Chlamydophila psittaci *em psitacídeos domiciliados em Rio Branco, Acre, Brazil*

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ABSTRACT: A large proportion of emerging infectious diseases (60.3%) globally are zoonotic pathogens, and of these, 71.8% originate from wild animals. Salmonellosis and psittacosis, diseases caused by *Salmonella* spp. and *Chlamydophila psittaci*, respectively, in wild animals are zoonoses with great risks to public health. Therefore, this study aimed to investigate the presence of *Salmonella* spp. and *C. psittaci* in parrots domiciled in Rio Branco, Acre. The animals in the study were raised as pets, and selection was performed based on convenience criteria. The birds were manually restrained to collect biological materials. Subsequently, conventional microbiological and biochemical tests were performed to identify *Salmonella* spp., and polymerase chain reaction analyses were conducted to identify *C. psittaci* and *Salmonella* spp. It was not possible to isolate *Salmonella* spp. and *C. psittaci* in the sampled birds. However, the presence of these bacteria in parrots cannot be ruled out because intermittent release and diagnostic limitations are widely described in the literature.

KEYWORDS: Birds; Chlamydiosis; Salmonellosis; Wild Animals.

RESUMO: Uma grande proporção das doenças infecciosas emergentes (60,3%) em todo o mundo são de patógenos zoonóticos e, destes, 71,8% se originam de animais selvagens. Salmonelose e psitacose, doenças causadas por *Salmonella* spp. e *Chlamydophila psittaci*, respectivamente, em animais silvestres são zoonoses com grandes riscos à saúde pública. Portanto, este estudo teve como objetivo investigar a presença de *Salmonella* spp. e *C. psittaci* em papagaios domiciliados em Rio Branco, Acre. Os animais do estudo foram criados como animais de estimação e a seleção foi realizada com base em critérios de conveniência. As aves foram contidas manualmente para coleta de material biológico. Posteriormente, testes microbiológicos e bioquímicos convencionais foram realizados para identificar *Salmonella* spp., E análises de reação em cadeia da polimerase foram realizadas para identificar *C. psittaci* e *Salmonella* spp. Não foi possível isolar *Salmonella* spp. e *C. psittaci* nas aves amostradas. No entanto, a presença dessas bactérias em psitacídeos não pode ser descartada porque a liberação intermitente e as limitações diagnósticas são amplamente descritas na literatura.

PALAVRAS-CHAVE: Animais Silvestres; Aves; Clamidiose; Salmonelose.

INTRODUCTION

Most emerging infectious diseases are caused by pathogens that cause zoonoses, which represent 60.3% of all emerging infectious diseases. Among zoonotic diseases, 71.8% originate from wild animals (JONES et al., 2008). Wild birds can harbor important zoonotic pathogens, and their involuntary displacement caused by trafficking is a key mechanism by which novel disease outbreaks may emerge in different locations (GIOVANINI, 2001).

Salmonellosis is caused by the enterobacterium *Salmonella*, which has high endemicity and morbidity and is very difficult to control (HOFER; FILHO; REIS, 1997). Its main route of transmission is oral and/or fecal, and transmission occurs indirectly through the ingestion of contaminated food or directly through contact with infected wild or domestic animals (ALLGAYER et al., 2009; MURRAY, 2000). It is also of great risk to public health because infected animals can spread this disease to

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other animals and humans without presenting clinical signs (Lima et al., 2012; MURRAY, 2000).

Raso et al. (2010) reported that psittacosis, also called avian chlamydiosis, caused by *Chlamydophila psittaci*, is one of the main zoonoses transmitted from birds to humans. The highest risk group comprises people who work directly with these animals and owners of pet birds. Therefore, according to the authors, psittacosis can be considered an occupational disease.

It is difficult to diagnose these diseases in parrots, as they have a slow evolution and often go unnoticed, thus making their treatment difficult. Its diagnosis is extremely important because the presence or of these agents are important health risk determinants in both wild and captive bird populations (TULLY; HARRISON, 1994).

Considering this background, the objective of this study was to detect the presence of *Salmonella* spp. and *C. psittaci* and determine the pathway of release of *C. psittaci* in parrots domiciled in Rio Branco, Acre, Brazil.

MATERIAL AND METHODS

Animals, selection location, and anamnesis

This study was approved by the Animal Use Ethics Committee of Federal University of Acre under case number 23107.019896 / 2017-64 and protocol number 40/2017. Parrots domiciled in urban and rural areas of Rio Branco, Acre, Brazil from December 2018 to October 2019.

Study animals were selected according to a convenience criterion in which the availability of families to participate in the collection of biological materials was considered. The birds had their data recorded and, to identify each animal's routine, facilities, sanitary management, feeding, and possible clinical treatments performed for each bird were also observed.

For this experiment, 100 parrots from 25 different owners were selected (Figure 1). Fecal and oral mucosa samples were collected for further analysis in the laboratory. The collection was performed following the guidelines of Benez (2004), which includes only necessary physical restraint with care to avoid compressing the animal's chest, ensuring normal breathing movements and avoiding asphyxiation.

Collection of biological materials

Soon after containment, biological material samples were collected. Two sterile swabs in the cloaca and two swabs of oral material were used; in total, four swabs were taken from each bird, with two designated for analyzing *Salmonella* spp. and two for analyzing *Chlamydophila psittaci*. The swabs were placed in test tubes, previously identified with the respective file number of each animal to ensure accurate identification of data for each bird.

Fecal and oral swabs were packaged and stored at –20 °C until they were sent to the Molecular Epidemiology Laboratory of the Department of Veterinary Public Health and Public Health of Federal Fluminense University for DNA and PCR extraction. The other samples were sent to the Microbiology Laboratory of

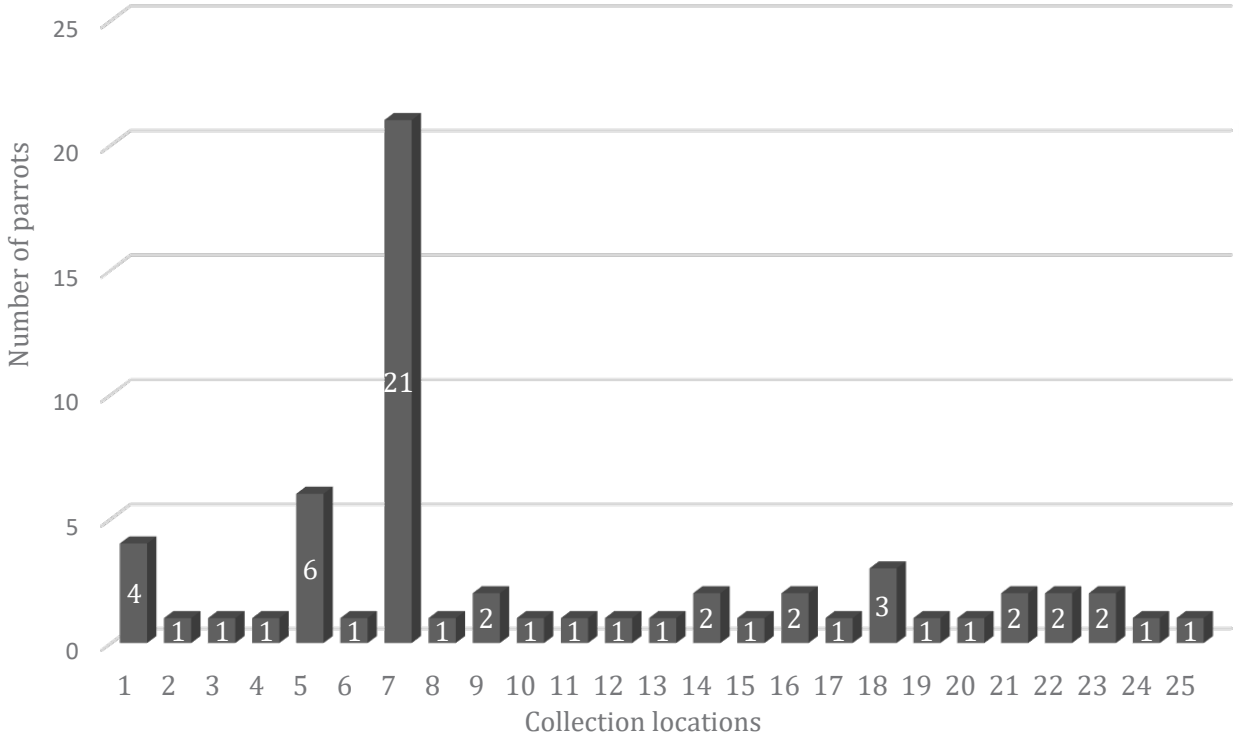

Figure 1. Locations and number of collected parrots in Rio Branco, Acre.

the Veterinary Medicine Unit of the Federal University of Acre for initial conventional bacteriological analysis.

For *Salmonella* spp., only cloacal samples were used, and for *C. psittaci*, both cloaca and oral samples were analyzed.

Laboratory processing for Salmonella spp. isolation

The collected material was kept in tubes and maintained in an oven at 36 °C for 24 h. After 24 h, the samples were homogenized and subjected to selective enrichment in Rappaport Vassiliadis broth at 42 °C for 24 h, according to Pickler et al. (2012) and Gargiulo et al. (2018). Samples obtained from selective enrichment were plated on bright green agar and xylose-lysine-deoxycholate agar, which were streaked to obtain isolated colonies. All plates were incubated and inverted at 37 °C for 24 h for subsequent isolation and agent identification, based on microscopic and macroscopic characteristics.

Colonies suggestive of *Salmonella* spp. were biochemically identified using Enterokit (PROBAC do Brasil Produtos Bacteriológicos Ltda. - São Paulo, Brazil) according to the manufacturer's standards.

For DNA extraction, the QIAamp Fast DNA Stool Mini Kit (QIAGEN, Valencia, CA, USA) was used according to the manufacturer's instructions. To demonstrate sample viability, quantification and purity analysis of the extracted DNA were performed in the UV range using a spectrophotometer.

Complementary diagnosis was conducted in the Molecular Epidemiology Laboratory of the Department of Veterinary Public Health and Public Health of Federal Fluminense University using polymerase chain reaction (PCR) according to Rahn et al. (1992). The primers used were (5'-GTGAAATTATCGCCACGTTCGGGCAA-3 ') and B (5'-TCATCGCACCGTCAAAGGAACC-3'). The positive control, a positive sample of *Salmonella* spp., was provided by the Molecular Epidemiology Laboratory of the Department of Collective Veterinary Health and Public Health of Federal Fluminense University, and ultrapure water was used as the negative control. A 5 µl aliquot of the reaction mixture was subjected to electrophoresis on an agarose gel and visualized under ultraviolet light.

Laboratory processing for C. psittaci isolation

For DNA extraction, the DNeasy Blood & Tissue Kit (QIAGEN, Valencia, CA, USA) was used according to the manufacturer's instructions. Oral material and cloacal sample analyses were conducted separately to determine the pathway of greater pathogenic release. To determine sample viability, quantification and purity analysis of the extracted DNA were performed in the UV range using a spectrophotometer.

PCR was performed according to the technique described by Denamur et al. (1991). The primers used were Gene MOMP (1050 bp), CTL (5'-CAA GAT TTT CTA GA (T/C) TTC AT (C/T) TTG TT-3 ') and CTU (5'-ATG AAA AAA AAA CTC TTG AAA TCG G-3 '). For the positive control, DNA extracted from a commercial vaccine for cats (Fel-OVax®Lv-K IV + Calicivax/owa, USA) was used, and ultrapure water was used as the negative control. 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

Of the 100 parrots studied, 68/100 (68%) were native fauna and 32/100 (32% were exotic fauna, with the species *Amazon ochrocephala* (30/100, 30%) being the most abundant. In the case of exotic species, *Nymphicus hollandicus* was the most abundant (17/32), followed by *Melopsittacus undulatus* (13/32).

Regarding the animals' diets, 41 birds had a human diet in their diet. With regard to habitat, 47% (47/100) were fully confined, 33% (33/100) were semi-confined, and 20% (20/100) were freely raised. Moreover, 19% (19/100) of parrots were in proximity to other animal species such as cats, dogs, and other domestic and/or free-living birds.

In terms of sanitary management, it was possible to identify satisfactory management because the proximity of the parrots to their owners yielded greater care due to bad smells and dirt in cages.

No clinical changes were observed in 85% (85/100) of the birds, and 15% (15/100) showed clinical signs such as apathy, feather fall, beak changes, fungi, self-draining, limb changes, stress, dyspnea, and anorexia. Only 3% (3/100) of the birds had undergone any veterinary treatment, and for 9% (9/100), some medication was provided.

Diagnostic tests for conventional microbiology and PCR did not positively identify *Salmonella* spp. Similarly, PCR did not positively identify *C. psittaci*. Therefore, it was not possible to define the pathway of greater pathogen release in domiciled parrots.

In a complementary examination (PCR), was detected the presence of *Mycoplasma* spp. in a bird with a clinical sign of dyspnea, which is a sign that is also observed in cases of infection by *Chlamydophila psittaci*.

DISCUSSION

Presence of Salmonella spp. in feces of domiciled parrots

The non-diagnosis of this bacterium, both by conventional microbiological methods and by PCR, was probably due to the following: a) no bacterial presence in studied animals, and b) subclinical infection as birds did not present any clinical signs at the time of collection, which may suggest that the infection and disease state, with a small elimination of the agent in the feces, is undetectable. It must be considered that the amount of *Salmonella* spp. in relation to other bacteria is much smaller when we observe the enteric microbiota (World Health

Organization, 2010), which complicates detection. Another important factor to consider is the intermittent release of the agent by the excreta, which could be absent at the time of collection, making isolation in the samples impossible at specific collection times, which has been observed in other parrot studies (ALLGAYER et al., 2008; HARCOURT-BROWN, 2010; LOPES et al., 2016).

Our results corroborate those of Sareyyüpoğlu et al. (2008), who conducted a study on pet birds of different species in Ankara, Turkey and found that only 3 (1.62%) of 185 fecal samples were positive to *Salmonella* spp. Corrêa et al. (2013), who studied *Salmonella* spp. in parrots, did not obtain any positive samples, and Murer et al. (2018) investigated *Salmonella* spp. in exotic and native Psittaciformes kept in captivity in the central region of Rio Grande do Sul, and obtained negative results. They used the same diagnostic techniques used in this research, conventional microbiological analysis, which is considered the gold standard test, and PCR.

One obstacle in the present research was the impossibility of carrying out repeat collections owing to a lack of owners' willingness to continue study participation, as they were afraid that we would confiscate the birds. Marietto-Gonçalves et al. (2010) demonstrated the importance of repeat collections for salmonellosis diagnosis due to its intermittent release. In the work of these authors, *S. enterica* was isolated, specifically the subspecies *S. enterica serotype Enteritidis*. From birds diagnosed with *Salmonella*, three were apparently healthy adults, which corroborates that wild animals are asymptomatic disseminators of this pathogen (Lima et al., 2012).

It is worth mentioning that, in its subclinical form, the elimination of the agent, as in *Salmonella*, is intermittent, which makes isolation more difficult (PROENÇA; FAGLIAR; RASO, 2011).

In a study by Tunca et al. (2012) in Turkey on budgerigars (*Melopsittacus undulatus*) from three commercial farms, the presence of *S. gallinarum* was detected. Compared with other species of birds, it presented a high mortality rate, which demonstrates the high susceptibility of these parrots to the studied pathogen. Contamination of captive birds usually occurs through contaminated food and water, contact with other birds, and contact with rodents, which are known sources of *Salmonella* contamination (ANDREATTI FILHO, 2007; FRIEND; FRASON, 1999; STEELE; GALTON, 1971). In the present study, it was observed that both *Melopsittacus undulatus* (13/32) and the other exotic species of parrots in their entirety were bred in total confinement, in contact with free-living birds and rodents. The cages where they were housed were mostly clean and well cared for owing to owner proximity.

It is also possible that the negative results in the samples of the parrots for *Salmonella* spp. in this study may be related to the sanitary management of cages, facilities, drinking fountains, and feeders, which can drastically decrease infection rate and risk (BERCHIERI; MACARI, 2000). It was observed that sanitary management was adequate, thus mitigating the accumulation of dirt that would favor bacterial growth.

Bezerra et al. (2013) analyzed samples from breeding sites and pet shops and found negative results regarding the presence of *Salmonella* spp. The explanation was the sanitary measures taken by the owners. Keen et al. (2007) also found similar results in research carried out in zoos in the United States, and the authors attributed these results to sanitary management and excellent facility conditions.

The low frequency of *Salmonella* spp. Isolation in wild birds has been observed for many years in several studies and was further demonstrated by Dorrestein et al. (1985), who performed 466 necropsies and analyzed 80 fecal samples from *Cacatuinae*, *Trichoglossinae*, and *Psittacinae*; this study found only one positive result in fecal samples and eight positive results from necropsies. Similarly, Corrêa et al. (2013) found no samples positive for *Salmonella* spp. in 44 samples from captive parrots. Gopee, Adesiyun, and Caesar (2000) reported that when compared to mammals and reptiles, birds raised in captivity have a relatively low frequency of *Salmonella* spp. isolation.

Presence of C. psittaci in fecal and oral material from domiciled parrots

When questioned, the owners did not report any previous infection; however, one animal presented with dyspnea (1/100, 1%) at the time of collection, a clinical sign that is consistent with the disease. *Chlamydophila psittaci* is always suspected in these cases, but in the differential examination, another pathogen, *Mycoplasma* spp., was observed. Failure to express clinical signs or expression of only advanced stages of the disease makes the clinical examination even more complex as the lack of clinical signs does not indicate the absence of infection (BRAZ et al., 2014).

In a survey of pet birds, Proença et al. (2010) found that 38% (35/92) of birds were positive for *C. psittaci*. Of these 35 birds, all exhibited or had a history of clinical signs of infection or were positive in routine tests (PCR); however, at the time of collection, these animals were clinically healthy. This was also observed in the present study, in which 85% (85/100) of the birds did not present any type of clinical alteration and 14% (14/100) showed non-specific clinical signs.

The form of sampling used in this study aimed to identify study animals without casuistry of the disease based on randomness with the goal of identifying possible avian *C. psittaci* carriers in proximity with humans. This method was also used by Silva (2013), who analyzed 85 cockatiels from farms and 21 birds from owners, isolated *C. psittaci* from only one bird, which originated from a farm rather than captivity (1/85; 1.2%). According to the author, one plausible explanation for the low isolation rate of *C. psittaci* was the

form of sampling used, in which randomness is necessary to avoid biased sampling.

However, it was observed that the majority of positive reports of *C. psittaci* in the literature came from studies with animals that were taken to veterinary clinics and hospitals with a characteristic symptom of disease development or those that previously had these symptoms. This was the case in the study by de Proença et al. (2010), who collected data from animals previously attended by veterinarians, selecting birds that had already tested positive for the presence of *C. psittaci* in routine PCR or that had clinical signs compatible with the disease. In such cases, there is a greater chance of bacterial isolation.

However, a key factor that can increase infection risk was considered in the selection of animals for this work, which is the proximity of birds to other animals, especially other captive or free birds. Of the 100 birds sampled, 11 had some kind of contact with other bird species. *Chlamydophila psittaci* infects not only Psittaciformes but also more than 30 orders of birds (KALETA; TADAY, 2003), which increases the possibility of infection after interaction, as seen by Proença et al. (2010), in which 85% of positive identification occurred in birds with a history of contact with other captive or free-living birds.

The sanitary management of bird cages and enclosures must also be considered as environmental and utensil cleaning and disinfecting measures help in controlling the disease (BERCHIERI; MACARI, 2000). In the present study, we observed satisfactory sanitary management by the owners, which may have limited bacterial proliferation. Araujo et al. (2019) reported the highest incidence of *C. psittaci* in places that received the worst assessments in terms of sanitation, leading to a greater number of cases. As already pointed out by Raso (2007) and Vasconcelos et al. (2013), strict cleaning, breeding site disinfection, quarantine, and migratory bird treatment are effective measures for controlling and reducing the presence and proliferation of the disease in the environment.

CONCLUSION

It was not possible to isolate *Salmonella* spp. and *C. psittaci* in the sampled birds. However, the presence of these bacteria in parrots cannot be ruled out because intermittent release and diagnostic limitations are widely described in the literature. In addition, the differential diagnosis of *Mycoplasma* spp. highlights the importance of health monitoring in wild birds raised as pets.

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