DETECTION OF ZOONOTIC AGENTS IN PIGEONS (*Columba livia*) FROM MACEIÓ, ALAGOAS

Artur Bibiano de Vasconcelos[1]
Danilo Alves de França[2]
Juliana Carla Cavalcanti Marques[3]
Karla Patrícia Chaves da Silva[3]

ABSTRACT

Due to the growing population of pigeons (*Columba livia*) in the Maceió and the lack of information regarding their importance in public health for the city, the aim was to detect the presence of agents with zoonotic potential in 30 animals of this species. The study was carried out with pigeons found in areas close to the main hospitals, squares and beaches. The pigeons were captured and transferred to individual cardboard boxes, where they remained for six hours to spontaneously defecate. Blood was collected using a hypodermic needle and the cloacal cavity were swabbed. The feces were used for bacterial isolation and fungi Isolation of the genera *Cryptococcus* and *Histoplasma*. The serum obtained after blood clotting was used in the Immunofluorescence Antibody Test (IFAT) to identify anti-*Toxoplasma gondii* antibodies. To investigate the presence of *Chlamydophyla psittaci*, cloacal secretions were used in diagnostic PCR. The results showed the isolation of six samples of *Cryptococcus* and two of *Histoplasma*. Different bacterial genera were detected from the cloacal mucosa samples, with *Staphylococcus* and *Streptococcus* being found most frequently, followed by Enterobacteriaceae, *Corynebacterium* and *Bacillus*. The *Staphylococcus* samples were 100% sensitive to all the antibacterials tested, but no antibiotic was effective for all the bacterial genera. *C. psittaci* DNA and anti-*T. gondii* antibodies were not detected in the samples. It can be concluded that the pathogens isolated are infectious and pose a serious health risk to people who circulate in these environments.

Keywords: zoonosis, fungi, diagnosis, *Cryptococcus*, *Histoplasma*.

DETECÇÃO DE AGENTES ZOONÓTICOS EM POMBOS (COLUMBA LIVIA) DE MACEIÓ, ALAGOAS

RESUMO

Devido ao crescimento da população de pombos (*Columba livia*) na cidade de Maceió e a ausência de informações referentes à sua importância em saúde pública para a cidade, objetivou-se detectar a presença de agentes com potencial zoonótico em 30 animais da espécie. O estudo foi realizado com pombos encontrados em áreas próximas aos principais hospitais, praças e praias. As aves foram capturadas e transferidas para caixas individuais de papelão, onde permaneceram por seis horas para a defecação espontânea. Colheu-se sangue com agulha hipodérmica e realizou-se suabe na cavidade cloacal. As fezes foram utilizadas para o isolamento bacteriano e isolamento fúngico de fungos dos gêneros *Cryptococcus* e *Histoplasma*. O soro obtido após a coagulação do sangue foi utilizado na Reação de Imunofluorescência Indireta (RIFI) para a identificação de anticorpos anti-*Toxoplasma gondii*. Para a investigação da presença de *Chlamydophyla psittaci*, utilizaram-se as secreções oral e

[1] São Paulo State University. artur.bibiano@unesp.br
[2] Universidade Estadual Paulista. *Correspondência. danilo.franca@unesp.br
[3] Federal University of Alagoas. juliana.marques@vicosa.ufal.br

cloacal em PCR-diagnóstico. Como resultados, observou-se o isolamento seis amostras de *Cryptococcus* e duas de *Histoplasma*. A partir das amostras de mucosa cloacal detectou-se diferentes gêneros bacterianos, sendo encontrados com maior frequência *Staphylococcus* e *Streptococcus*, seguido de Enterobacteriaceae, *Corynebacterium* e *Bacillus*. As amostras de *Staphylococcus* foram 100% sensíveis para todos os antibacterianos testados, porém nenhum antibiótico apresentou eficácia para todos os gêneros bacterianos. Nas amostras não houve detecção de DNA de *C. psittaci* e de anticorpos anti-*T. gondii*. Conclui-se que os patógenos isolados são infectocontagiosos, representando um sério risco para a saúde de pessoas que circulam nesses ambientes.

Palavras-chave: Zoonose, Fungos, Epidemiologia, *Cryptococcus*, *Histoplasma*.

DETECCIÓN DE AGENTES ZOONÓTICOS EN PALOMAS (COLUMBA LIVIA) DE MACEIÓ, ALAGOAS

RESUMEN

Debido a la creciente población de palomas (*Columba livia*) en la ciudad de Maceió y a la falta de información sobre su importancia en salud pública para la ciudad, el objetivo fue detectar la presencia de agentes con potencial zoonótico en 30 palomas. El estudio se realizó con palomas encontradas en áreas próximas a los principales hospitales, plazas y playas. Las aves fueron capturadas y transferidas a cajas de cartón individuales, donde permanecieron durante seis horas para defecar espontáneamente. Se recogió sangre con una aguja hipodérmica y se tomaron hisopos de la cavidad cloacal. Las heces se utilizaron para el aislamiento bacteriano y aislamiento de hongos de los géneros *Cryptococcus* e *Histoplasma*. El suero obtenido tras la coagulación de la sangre se utilizó en la reacción de inmunofluorescencia indirecta (RIFI) para identificar anticuerpos anti-*Toxoplasma gondii*. Para investigar la presencia de *Chlamydophyla psittaci*, se utilizaron secreciones cloacales en el diagnóstico por PCR. Los resultados mostraron el aislamiento de seis muestras de *Cryptococcus* y dos de *Histoplasma*. En las muestras de mucosa cloacal se detectaron diferentes géneros bacterianos, siendo *Staphylococcus* y *Streptococcus* los más frecuentes, seguidos de Enterobacteriaceae, *Corynebacterium* y *Bacillus*. Las muestras de *Staphylococcus* fueron 100% sensibles a todos los antibacterianos probados, pero ningún antibiótico fue eficaz para todos los géneros bacterianos. No se detectó DNA de *C. psittaci* ni anticuerpos anti-*T. gondii* en las muestras. Se concluye que los patógenos aislados son infecciosos y suponen un grave riesgo para la salud de las personas que circulan por estos ambientes.

Palabras clave: zoonosis, hongos, epidemiología, *Cryptococcus*, *Histoplasma*.

INTRODUCTION

The presence of pigeons in large urban centers represents an emerging risk for the transmission of serious infectious and parasitic diseases. These pigeons are hosts to a rich diversity of bacteria, fungi and parasites, and through their secretions and excretions, they spread these agents in the environment, representing a risk to public health (1,2).

Due to the growth of the pigeon population and the lack of information regarding their importance in the transmission of diseases in the Maceió city, the aim was to detect the presence of agents with zoonotic potential in this species, such as *Cryptococcus*, *Histoplasma* and *Chlamydophyla psittaci*, detect the presence of anti-*Toxoplasma gondii* antibodies and isolate bacteria from the cloacal cavity, as well as assessing their in vitro sensitivity.

MATERIAL AND METHODS

All the research procedures were carried out with the approval of the Ethics Committee for the Use of Animals (CEUA) of the Federal University of Alagoas, protocol number 20/2013.

The study was carried out with birds present in areas close to hospitals, urban squares and beaches in the municipality of Maceió, capital of the state of Alagoas. Samples were collected from 30 pigeons. Using ground corn as bait, the birds were captured in wire traps and stored in individual cardboard boxes with foil-lined bottoms, where they remained for six hours for spontaneous defecation and sample collection. Afterwards, the birds were released at the place of capture and the samples were taken for processing at the Laboratory of Veterinary Microbiology of the Federal University of Alagoas.

For the identification of *Cryptococus* spp. and *Histoplasma* spp., fecal samples were used from the captured animals. The samples were macerated in test tubes containing 9 mL of saline solution plus antibiotic (amoxicillin 500mg) and incubated for 24 hours at 37ºC. They were then inoculated in duplicate onto Petri dishes containing Sabouraud agar and sheep's blood agar to grow the fungi in filamentous and yeast form, for 14 days (room temperature) and 48 hours (37ºC), respectively. The imprint technique was used to identify the filamentous fungi stained with methylene blue and viewed under 40x optical microscopy. To identify the yeasts, slides were made and stained using the Gram technique and viewed under 100x immersion light microscopy. Fungal identification was based on morphological and phenotypic characteristics using biochemical tests.

In order to detect *Chlamydophyla psittaci*, samples of oral and cloacal secretions were used, acquired using swabs and kept frozen until the diagnostic test was carried out. For identification, DNA was first extracted directly from the secretions using the Illustra Tissue and Cells Genomic Prep Mini Spin Kit (GE Healthcare Life Sciences, Marlborough, MA, USA), following the protocol provided by the manufacturer. The extracted material was then amplified using a Nested-PCR (3) with the primers cla420 (5'CAG GAC ATC TTG TCT GGC TTT AA-3') and cla422 (5'-GCA AGG ATC GCA AGG ATC-3') and cla421 (5'-TTA GAG GTG AGT ATG AAA AAA CTC-3'). Five microliters of the PCR product was processed in a 1.5% agarose gel at 100 V. To establish molecular weight standards, DNA Ladder 100 bp (Invitrogen, Waltham, MA, USA) was used to visually assess the sizes of the amplified fragments. The gel was visualized under ultraviolet light (Bio-Rad, Hercules, CA, USA) using Image Lab software version 4.1.

Fecal samples were used for bacterial microbiota identification and antibiogram. The material was sown using the platinum loop depletion technique in Petri dishes containing 10% sheep blood agar medium and McConkey agar medium. They were then incubated in a bacteriological oven for 48 hours in an aerobic environment at 37°C. The samples were subjected to observation of the macroscopic characteristics of the colonies, and microscopic characteristics of the agent, using Gram staining, viewed through a 100x objective in an optical microscope. Gram-negative bacteria were identified by biochemical tests. After identification, the bacteria were subjected to an antibiogram using the disk diffusion technique containing different antibiotics. The antibiotics used were Penicillin (10 IU), Vancomycin (30 µg), Chloramphenicol (30 µg) and Tetracycline (30 µg). The plates were kept in a bacteriological oven for 24 hours at 37ºC. After the incubation period, the bacterial growth inhibition halos were measured for each antimicrobial (4).

Serum samples were tested for the presence of anti-*T. gondii* antibodies using the Immunofluorescence Antibody Test (IFAT) (5). Specific IgG anti-pigeon conjugate antibodies were used. Each serum sample was diluted in 0.01M phosphate buffered saline (PBS) pH7.2 at dilutions of 1:16, 1:64 and 1:256. In the same microplate, the same process was carried out for

the positive and negative control sera, which were supplied by the Microbiology Laboratory, Viçosa, Brazil, from pigeons previously diagnosed by the service. The sera in their respective dilutions were applied to the previously sensitized slides and incubated at 37°C for 30 minutes. After washing in PBS 7.2, a commercial anti-pigeon IgG antibody conjugated with fluorescein isothiocyanate (Bethyl Laboratories, Montgomery, TX, USA) was applied to the slides, serving as a secondary antibody in the reaction. This conjugate was diluted according to its previously established titer in a 20 mg% Evans blue solution. The slides were incubated again at 37°C for 30 minutes. After the slides had dried, they were read under an immunofluorescence microscope with a 40x objective, considering the end point of the reaction to be the highest dilution of serum in which there was still complete and intense fluorescence at the edge of at least 50% of the tachyzoites.

RESULTS AND DISCUSSION

After analysis, six colonies of *Cryptococcus* spp. were identified. The yeasts were visible as globose, rounded, regular, encapsulated and without hyphae, as shown in Figure 1. The prevalence of the fungus in the samples was 20% (6/30). A similar result was observed in Lages, Santa Catarina state, where 58 fecal samples were analyzed from the soil of pigeons in the city's most popular squares. Of the samples analyzed in this study, 25.86% (15/58) were positive (6). In the city of Pelotas, Rio Grande do Sul state, the isolation of *Cryptococcus* occurred in 26.9% (7/26), where pigeon droppings were collected from buildings, squares and outdoor locations in the city (7). The number of studies involving this fungus in pigeon samples in Brazil is scarce.

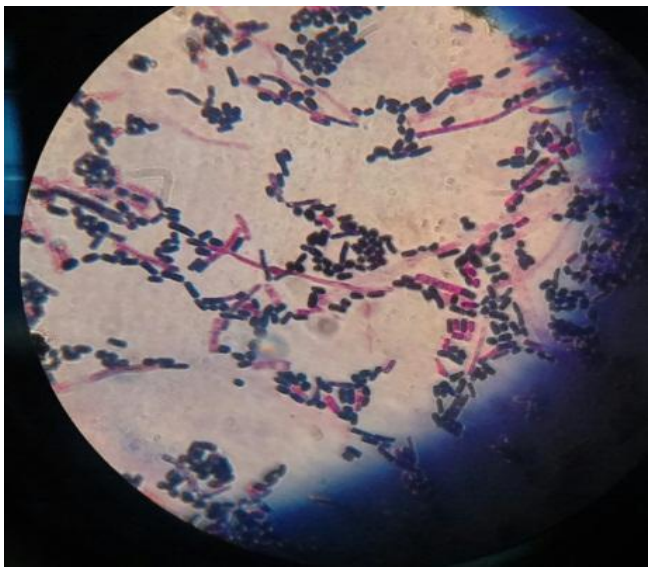

Figure 1. Colony of *Cryptococcus* spp. analyzed under light microscopy. The yeasts are globose, rounded, regular, encapsulated and without hyphae.

Two *Histoplasma* spp. colonies were identified based on their morphological and phenotypic characteristics, where in their filamentous form, they were white in color with a cottony appearance, as shown in Figure 2. Microscopy revealed the presence of delicate, septate hyphae with microconidia and macroconidia. In yeast form, the colonies were creamy and moist. Under microscopy, they were oval and some showed budding. Thus, the prevalence of *Histoplasma* was 6.67% (2/30). The identification of these fungi reveals the risk to the human population (8).

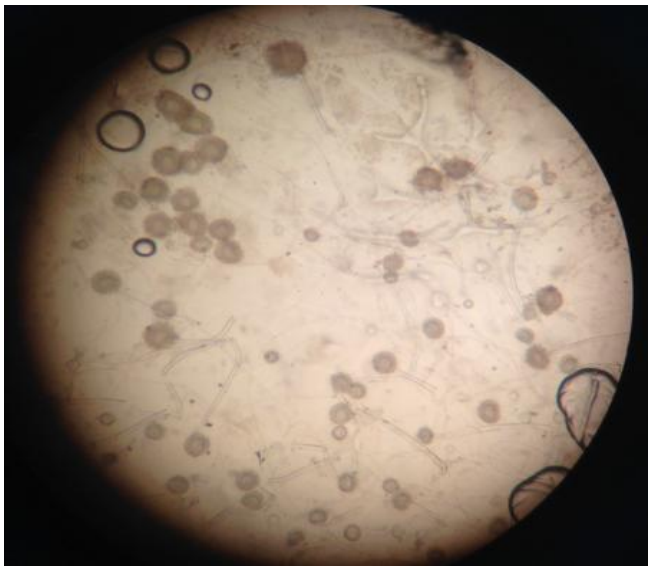

Figure 2. Colony of *Histoplasma* spp. analyzed under light microscopy. The yeasts are white, creamy, moist, oval-shaped and some show budding.

C. psittaci was not detected in any of the samples. In a study carried out in Poland, the presence of *C. psittaci* was investigated using the real-time PCR technique in free-living pigeons and detected in 3.5% (5/143) of the cloacal swab samples (9).

In the bacterial isolation, different bacterial genera were observed in the cloacal mucosa of the pigeons. The bacteria found most frequently were *Staphylococcus* (70%; 21/30) and *Streptococcus* (70%; 21/30). This was followed by Enterobacteriaceae (50%; 15/30), *Corynebacterium* (40%; 12/30) and *Bacillus* (40%; 12/30). The genera *Staphylococcus* and *Streptococcus* belong to the skin and mucous membrane microbiota of mammals and birds and can be transmitted by saliva and contaminated aerosols. Bacteria belonging to the Enterobacteriaceae family represent a risk in food contamination, are highly virulent bacteria and can produce serious diseases. These agents isolated from the oral mucosa of pigeons represent a risk to public health. A high antimicrobial sensitivity pattern was detected in the bacteria from the mouth mucosa of pigeons. The *Staphylococcus* samples were 100% sensitive to all the antibacterials tested, followed by Enterobacteriaceae 66.67%, *Corynebacterium* 66.67% and *Streptococcus* 50%. The bacteria present in the oral mucosa of pigeons already have species that are resistant to antimicrobials; in this study, no antibiotic was effective for all the bacterial genera. Pigeons acquire these bacteria, probably from the environment and food contaminated by resistant microorganisms (10).

No anti-*T. gondii* antibodies were detected. However, it is worth noting that free-living birds have the ability to explore large areas and thus present a greater risk of exposure to T. gondii infection, and are considered good indicators of environmental contamination (11-13). In addition, birds are considered important reservoirs of the parasite, commonly becoming sources of infection for definitive hosts (cats), due to the predatory and carnivorous nature of these animals (14).

Important bacterial agents with zoonotic potential have been identified in animal clinical samples from Alagoas State in recent years, alerting local health services to the occurrence of these diseases (15-17).

The agents isolated are infectious and represent a serious health risk for people who circulate in public environments and a risk factor for the transmission of hospital-acquired infections to patients already in the health unit, thus requiring the implementation of pigeon control measures in the city, with emphasis on the regions studied. In addition, the species isolated in this study are resistant to antimicrobials, with no antibiotic showing efficacy for all

bacterial genera, indicating the need to carry out antibiogram tests before starting treatment for bacterial zoonoses transmitted by pigeons.

CONCLUSIONS

From the cloacal mucosa samples, different bacterial genera were detected, with *Staphylococcus* and *Streptococcus* being found most frequently, followed by Enterobacteriaceae, *Corynebacterium* and *Bacillus*. The *Staphylococcus* samples were 100% sensitive to all the antibacterials tested, but no antibiotic was effective for all the bacterial genera. *C. psittaci* DNA and anti-*T. gondii* antibodies were not detected in the samples. *Cryptococcus* and *Histoplasma* fungi were isolated and identified. The pathogens isolated are infectious and represent a serious health risk for people who circulate in these environments.

REFERÊNCIAS

1. Mota RA, Silva KPC, Freitas MFL, Porto WJN, Silva LBG. Utilização indiscriminada de antimicrobianos e sua contribuição a multirresitência bacteriana. Brazilian Journal of Veterinary Research and Animal Science, 2005; 42(6):465-470.

2. Cades M, Zanini DS, Souza HL, Silva JD, Fink AAB, Muniz IM. Perfil de resistência antimicrobiana de mastite bovina em propriedade leiteira no município de Monte Negro/RO. Revista Brasileira de Ciências da Amazônia/Brazilian Journal of Science of the Amazon, 2017;6(1):15-20.

3. Buxton D, Rae, AG, Maley SW, Thomson KM, Livingstone M, Jones GE, Herring AJ. Pathogenesis of Chlamydia psittaci infection in sheep: detection of the organism in a serial study of the lymph node. Journal of comparative pathology, 1996;114(3):221-230.

4. CLSI. Performance standards for antimicrobial susceptibility testing. Fifteenth informational supplement, m100-s15, Clinical and Laboratory Standards Institute, Wayne, 2005.

5. Pinto GLB, Santarém VA, Ribeiro J, Filho RTS, França DA, Moraes GN, et al. Toxoplasmosis Behind Bars: One Health Approach on Serosurvey Dynamics and Associated Risk Factors for Women Inmates, Correctional Officers, and In-Prison Feral Cats. Transboundary and Emerging Diseases, 2024; 2024(1):1-9.

6. Scain, G. Prevalência de *Cryptococcus neoformans* em fezes de pombos (*Columba livia*) nas praças públicas da cidade de Lages, Santa Catarina, 2011.

7. Faria ROD, Nascente PDS, Meinerz ARM, Cleff MB, Antunes TDA, Silveira É. DS, Mello JRBD. Ocorrência de *Cryptococcus neoformans* em excretas de pombos na cidade de Pelotas, Estado do Rio Grande do Sul. Revista da Sociedade Brasileira de Medicina Tropical, 2010;43:198-200.

8. Araúz AB, Papineni P. Histoplasmosis. Infect Dis Clin North Am. 2021;35(2):471-491.

9. Kowalczyk K, Wójcik-Fatla A. *Chlamydia psittaci* in Faecal Samples of Feral Pigeons (*Columba livia*) in Urban Areas of Lublin city, Poland. Curr Microbiol. 2022;79(12):367.

10. Grond K, Perreau JM, Loo WT, Spring AJ, Cavanaugh CM, Hird SM. Longitudinal microbiome profiling reveals impermanence of probiotic bacteria in domestic pigeons. PLoS One. 2019;14(6):e0217804.

11. Dubey JP, Murata FHA, Cerqueira-Cézar CK, Kwok OCH, Su C. Epidemiologic significance of *Toxoplasma gondii* infections in turkeys, ducks, ratites and other wild birds: 2009-2020. Parasitology. 2021;148(1):1-30.

12. Khordadmehr M, Sazmand A, Almasi P, Shahbazi P, Ranjbar V, Otranto D, Hemphill A. Natural infection with *Toxoplasma gondii*, *Neospora caninum* and *Sarcocystis* species in domestic pigeons (*Columba livia*) in Iran. Comp Immunol Microbiol Infect Dis. 2023;93:101946.

13. Karadjian G, Laboutière L, Chevillot A, Voisinot A, Blaizot A, Puech MP, Aubert D, Risco-Castillo V, Blaga R, Vallée I. *Toxocara cati* and *Toxoplasma gondii* in French Birds of Prey. J Wildl Dis. 2022;58(2):373-379.

14. Panazzolo GK, Kmetiuk LB, Domingues OJ, Farinhas JH, Doline FR, França D. AD, Fávero GM. One Health approach in serosurvey of *Toxoplasma gondii* in former black slave (Quilombola) communities in southern Brazil and among their dogs. Tropical Medicine and Infectious Disease, 2023;8(7):377.

15. Vasconcelos AB, Andrade VD, Moraes ACID, Ramos EMOA, Silva ACA, França DAD, Silva KPC. Occurrence and antimicrobial resistance profile of *Salmonella* spp. in calves from the Mesoregion Sertão of Alagoas, Brazil. Acta Vet Bras, 2021:36-40.

16. Vasconcelos AB, Andrade V, França DA, Dantas AA, Silva TBC, Silva KPC. Inibição in vitro de *Salmonella* spp. Isolada de bezerros do sertão alagoano com extratos etanólicos de plantas da mata atlântica. Veterinária e Zootecnia, 2022;29:1-7.

17. Vasconcelos AB, França DA, Ferreira MDN, Araújo DK, Vasconcelos CB, Silva KPC. Detection of *Salmonella* spp. in eggs for human consumption from poultry farms in municipalities of Alagoas State, Brazil. Acta Veterinaria Brasilica, 2022;16(3).

Recebido em: 15/02/2024
Aceito em: 04/09/2024