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SHORT COMMUNICATION

Molecular detection of haemobacteria in Colombian wild birds

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ABSTRACT. Colombia shows a high density and variety of bird species, making it one of the most diverse avian territories globally. Antioquia ranks among the top four provinces with the greatest variety of bird species, underscoring the importance of research efforts on the local bird fauna. Therefore, this study aimed to identify bacterial agents in the blood of wild birds from the municipality of Jardín (Antioquia, Colombia) by 16S qPCR sequencing. A descriptive cross-sectional study was conducted using non-probabilistic convenience sampling. Wild birds were captured using mist nets and blood samples were collected from each animal via puncture using sterile lancets in the brachial vein, and a drop of blood was collected on filter paper for qPCR analysis. The 16S gene in bacterial genomes was found in 13 out of 46 wild birds of the Passeriform and quasi-Passeriform orders, captured at three different locations within the study municipality at altitudes ranging from 1,665 to 2,034 m.a.s.l. Seven different bird species were recorded and four different haemobacteria were identified (i.e. Exiguobacterium spp., Escherichia coli, Stenotrophomonas spp., and Stenotrophomonas maltophilia). This study contributes to the knowledge in Colombia by identifying four different hemobacteria in wild birds. Further research is required on the health status of these birds and the attributable impacts on their populations and other related factors, including humans.

Keywords: Blood, *Exiguobacterium* spp., *Escherichia coli*; hemobacteria; *Stenotrophomonas* spp.; wild bird.

INTRODUCTION

Colombia, with its remarkable avian biodiversity, ranks among the most bird-diverse countries in the world. The province of Antioquia is one of the regions with the highest diversity of bird species, making essencial the study of the local avifauna (Vélez *et al.*, 2021). However, the high density of avian populations poses potential risks for the transmission of zoonotic diseases, highlighting the importance of the One Health approach. One Health emphasises the interconnectedness of human, animal, and environmental health, recognising that many diseases, particularly zoonoses, arise from human interactions with wildlife (Mackenzie & Jeggo, 2019). This interconnectedness is especially relevant in regions such as Colombia, where urban expansion and habitat conversion bring humans, domestic animals, and wildlife into increasingly close contact.

The risk of zoonotic bacterial transmission from wild birds is significant, particularly in areas in which human activity has altered the natural environment. Wild birds can carry a range of pathogens, including zoonotic bacteria, which may affect both humans and animals. Among these pathogens are *Escherichia coli*, *Salmonella* spp., Mycobacteria, *Pasteurella multocida*, and *Chlamydia psittaci*, which are known to cause severe illnesses in birds and can sometimes infect humans or domestic animals. *Escherichia coli*, that can cause septicemia, and diarrhea, respiratory distress, and lethargy (Bélanger *et al.*, 2011; Knöbl *et al.*, 2011; Borges *et al.*, 2017); *Salmonella* spp. which can lead to septicemia, and enteritis and weight loss (Pennycott *et al.*, 2006; Dos Santos *et al.*, 2020); Mycobacteria causing avian tuberculosis, which results in chronic illness and can be fatal due to weight loss, diarrhea, and respiratory issues (Gaukler *et al.*, 2009); *P. multocida*, responsible for fowl cholera, leading to acute septicemia or chronic infections in birds, causing from sudden death to chronic respiratory problems and joint infections (El-Demerdash *et al.*, 2023; Hashish *et al.*, 2023); and *C. psittaci* that can cause respiratory distress, diarrhea, and lethargy in birds, being also zoonotic (Liu *et al.*, 2019; Amery-Gale *et al.*, 2020; Stokes *et al.*, 2020).

The increasingly frequent interactions among humans, livestock, and wildlife in regions such as Antioquia increase the risk of zoonotic disease outbreaks, underlining the im-

portance of continuous monitoring and research on local bird populations. Understanding the prevalence of these bacteria in wild bird species is crucial not only for conservation efforts but also for public health and the economy. By monitoring the spread of these bacteria, conservationists and health professionals can work together to mitigate the risk of zoonotic transmission and protect both biodiversity and human health (Levin & Parker, 2012).

Given these challenges, employing a One Health approach—integrating the environmental, veterinary, and human health sectors—is essential. Such an approach enables early detection of zoonotic pathogens and helps prevent outbreaks that could impact wildlife conservation, public health, and the poultry industry (Chan *et al*., 2013; Naqvi *et al*., 2017). Therefore, this study sought to identify bacterial agents in wild bird in Jardín Antioquia) using molecular techniques to contribute to both wildlife conservation and public health efforts.

MATERIALS AND METHODS

Study area and bird sampling

A total of 46 wild birds of the Passeriform and quasi-Passeriform orders were captured in a natural area corresponding to a tropical montane cloud forest at six different locations within the study municipality at altitudes ranging from 1,665 to 2,053 m.a.s.l. in the municipality of Jardín (Southwest Antioquia, Colombia). Twenty different bird species were recorded (i.e. *Sporophila nigricollis*, *Myiozetetes similis*, *Zonotrichia capensis*, *Zimmerius chrysops*, *Stilpnia vitriolina*, *Thraupis episcopus*, *Tangara gyrola*, *Melanerpes rubricapillus*, *Tangara arthus*, *Eubucco bourcierii*, *Streptopelia decaocto*, *Atlapetes albinucha*, *Chlorophanes spiza*, *Momotus aequatorialis*, *Molothrus bonariensis*, *Ramphocelus dimidiatus*, *Elaenia flavogaster*, *Euphonia laniirostris*, *Elaenia frantzii*, and *Diglossa sittoides)*. Sampling was carried out for 2 weeks (17 July to 30 July 2022).

The capture processes followed the methodologies proposed by Ralph *et al*. (1996) and Álvarez *et al*. (2004), using monofilament nylon mist nets. After capture, the birds were sheltered in capture bags, where they remained for a maximum of 20 min post-capture. Subsequently, each bird was identified according to Hilty and Brown (2021), and Remsen *et al*. (2022).

Blood samples were collected from the brachial vein posterior to the left wing of each bird after cleaning with antiseptic alcohol. A puncture was made using sterile lancets, collecting a drop of blood on grade 3HW filter paper (65 g/m2) inside a Ziplock bag with a silica gel bead. It was immediately verified that each bird was in the condition to fly, and thereafter, it was released and returned to its habitat. The samples were labelled with consecutive numbers, species names, and identification of the study area, and transported to the laboratory where they were refrigerated at 4°C until analysis on 23 October 2022.

DNA extraction, 16S amplification, and sequencing

Two portions were extracted from each filter paper sample using a small hole punch. These portions were centrifuged with a PBS wash at 41,000 rpm × 120 min, and the supernatant was removed. The resulting sediment was resuspended in 450 µL of PBS. Total genomic DNA was extracted using the Blood or Body Fluids Spin protocol (QIAmp DNA Extraction Mini Kit ®, Qiagen, Germantown, MD), according to the manufacturer's instructions. The extracted DNA was quantified using spectrophotometry (Nanodrop), ensuring a concentration of at least 50 ng/µL, according to the method described by Xu *et al.* (2020).

The genetic material extracted from blood samples was subjected to qPCR for the detection of intraerythrocytic hemobacteria using specific primers (see Data availability statement) targeting 600 to 800 bp fragments of conserved regions of the 16S gene in bacterial genomes. Positive controls were provided by TestMol S.A.S laboratory, and DNase-RNase-free sterile water (Cat No.: 129114, Qiagen, Germany) was used as a negative control. Specific primers for Cytochrome B genes in mammals were used as internal controls for DNA extraction and qPCR (Pfeiffer *et al*., 2004). The use of these controls not only helped prevent contamination and ensure the validity of the results but also allowed for the replication of the experiment in future studies.

The qPCR assay was performed in a Mic qPCR Cycler 4 channel (BioMolecular Systems, Australia) using protocols standardised by the laboratory. MasterMix for real-time PCR (SYBR Green, Thermo Fisher Scientific®) was used with a final volume of 19 µL, including 5 µL of DNA. The thermal profile was run with an initial denaturation of 3 min at 95°C, followed by 35 cycles of 30 s at 95°C, 1 min at 57°C, and 1 min at 72°C, and a final extension for 5 min at 72°C.

The amplification products were sent to the National Center for Genomic Sequencing (Macrogen®, Korea) for DNA purification and sequencing.

Molecular data analyses

A taxonomic name was designated for each sequence using BLAST (Altschul *et al*., 1990). To construct and edit the phylogenetic trees, sequences were aligned using ClustalW (Higgins *et al*., 1992) in BioEdit (Hall, 1999) to derive the consensus sequence. Phylogenetic trees were constructed using the Neighbor-Joining method (Saitou & Nei, 1987) based on evolutionary distances computed via the Composite Maximum Likelihood (ML) method. MEGA version X (Kumar *et al*., 2018) was employed to perform both the phylogenetic and molecular evolutionary analyses. To refine the tree, the best-fit substitution model was evaluated as TN93 + I (Tamura & Nei, 1993) using the Find Best Fit substitution model tool. The robustness of the phylogenetic tree was tested using bootstrap analysis with 1,000 replicates, providing a measure of support for branching patterns. The resulting trees were visually edited and refined using MEGA X software to ensure clarity and accuracy.

RESULTS AND DISCUSSION

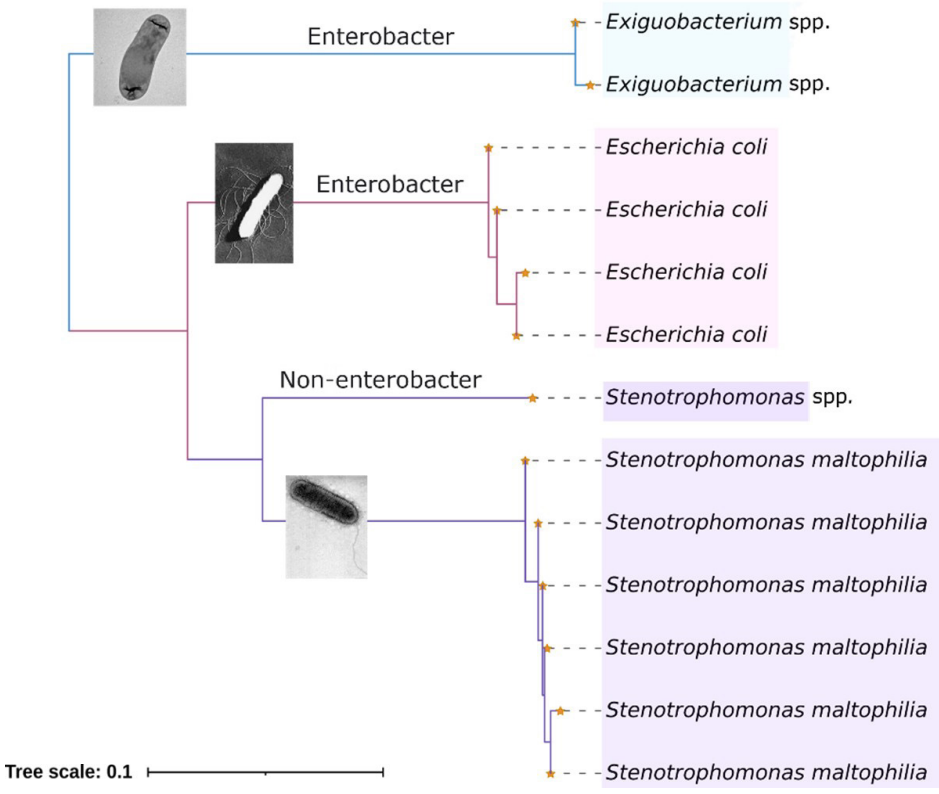
The objective of this study was to identify bacterial agents in the blood of wild birds through molecular approaches in an important area of native birds in Colombia. This area is renowned for its variety of ecosystems, and apart from threats linked to habitat, local bird populations might face susceptibility to infectious diseases, potentially exacerbating the challenges they face.

The 16S gene in bacterial genomes was found in 13 of 46 wild birds of the Passeriform and quasi-Passeriform orders. Positive birds were captured at three different locations within the study municipality, at altitudes ranging from 1,665 to 2,034 m.a.s.l. Four different haemobacteria were identified (i.e. *Exiguobacterium* spp., *E. coli*, *Stenotrophomonas* spp., and *Stenotrophomonas maltophilia*) (Figure 1) in seven different bird species recorded, with five adults and one juvenile (when possible, to determine the age group), as well as one male and one female (when possible, to determine the sex) (Table 1). No bird was recaptured.

Stenotrophomonas maltophilia, known for its pathogenicity in humans, can also impact wildlife, including wild birds. Affected individuals exhibit a range of symptoms including pneumonia, septicaemia, encephalitis, endocarditis, suppurative lymphadenitis, abscesses, and other disease syndromes (Brooke, 2012; Adegoke *et al*., 2017). This bacterium displays an open pan-genome, indicating extensive genetic variability across isolates from diverse environments (Xu *et al*., 2023). In South America, particularly in Peru, studies have identified multiple genotypes with varying resistance profiles, including significant resistance to trimethoprim/sulfamethoxazole and ceftazidime (Toledano *et al*., 2023) and to β-lactams and aminoglycosides in China (Li *et al*., 2023; Xu *et al*., 2023). This multidrug-resistant bacterium is also an opportunistic pathogen found in various environments such as water, rhizospheres, and other animals (Adegoke *et al*., 2017; Brooke, 2021). It possesses virulence factors, such as biofilm formation, motility, and antimicrobial resistance mechanisms, making it a concern for susceptible populations, including wild birds. In addition, studies have highlighted the ability of this bacterium to interact with other microorganisms, indicating its potential impact on diverse ecosystems (Brooke, 2021). While the research primarily focuses on clinical and environmental sources, the interaction of *S. maltophilia* with wild birds specifically is not directly addressed in the provided contexts. However, considering the species' adaptability and diverse

Table 1.
Characterization of the 13 study birds with positive molecular results to 16S gene of haemobacteria.

Cons.	Hemobacteria identified (% of compatibility in BLAST)	Altitude (m.a.s.l.)	Scientific name	Common name (in Spanish)	Common name (in English)	Sex	Age group
1	*Stenotrophomonas maltophilia* (100%)		*Stilpnia vitriolina*	Tángara rastrogera	Scrub tanager	ND	Adult
2	*Stenotrophomonas* spp. (100%)		*Thraupis episcopus*	Azulejo común	Blue-gray tanager	ND	Adult
3	*Stenotrophomonas* spp. (100%)		*Zimmerius chrysops*	Atrapamoscas caridorado	Golden-faced tyrannulet	ND	ND
4	*Stenotrophomonas maltophilia* (99%)	1,655	*Zonotrichia capensis*	Gorrión de montaña	Rufous-collared sparrow	ND	Juvenile
5	*Stenotrophomonas maltophilia* (97%)		*Tangara gyrola*	Tángara cabecirufa	Bay-headed tanager	ND	ND
6	*Stenotrophomonas maltophilia* (99%)		*Zimmerius chrysops*	Atrapamoscas caridorado	Golden-faced tyrannulet	ND	ND
7	*Escherichia coli* (97%)		*Zonotrichia capensis*	Gorrión de montaña	Rufous-collared sparrow	Male	ND
8	*Exiguobacterium* spp. (92%)		*Tangara arthus*	Tángara dorada	Golden tanager	ND	Adult
9	*Exiguobacterium* spp. (92%)	1,760	*Tangara gyrola*	Tángara cabecirufa	Bay-headed tanager	ND	ND
10	*Stenotrophomonas maltophilia* (99%)		*Eubucco bourcierii*	Torito	Red-headed barbet	Female	Adult
11	*Escherichia coli* (100%)		*Tangara arthus*	Tángara dorada	Golden tanager	ND	ND
12	*Escherichia coli* (97%)	2,034	*Thraupis episcopus*	Azulejo común	Blue-gray tanager	ND	Adult
13	*Escherichia coli* (100%)		*Stilpnia vitriolina*	Tángara rastrogera	Scrub tanager	ND	ND



Notes: Own work

Figure 1.
Phylogenetic tree of 16S nucleotide sequences (600-700 bp) obtained from the blood of wild birds. All ambiguous positions were removed for each pair of sequences (pairwise deletion option).

habitats, further investigation into the potential role of wild birds in the transmission or ecology of *S. maltophilia*, as well as the genus *Stenotrophomonas*, is mandatory.

Escherichia coli, a common bacterium in the gastrointestinal tract of warm-blooded animals, including wild birds, can significantly affect the gut microbiome. *E. coli* strains exhibit diverse circulating genotypes in South America and globally, with significant implications for public health. Recent studies have highlighted the prevalence of multidrug-resistant strains and their transmission pathways across various environments. Extended-spectrum β-lactamase (ESβL)-producing *E. coli*, particularly ST10, has been identified in South American llamas, indicating a global spread of these clones from wildlife to humans (Cárdenas-Arias *et al.*, 2023). In Bolivia, enterotoxigenic *E. coli* (ETEC) strains, notably ST218 and ST410, have been found in both clinical cases and environmental samples, emphasising the role of contaminated water as a reservoir for pathogenic strains (Calderón-Toledo *et al.*, 2023). Andean condors have been found to harbour critical priority *E. coli* strains with extensive resistance profiles, linking wildlife to the dissemination of antimicrobial resistance genes in ecosystems (Fuentes-Castillo *et al.*, 2020). Furthermore, the detection of critically important antimicrobial drug-resistant *E. coli* in various bird species suggests a potential for interspecies transmission of resistance, emphasising the need for increased research to understand the dynamics of drug-resistant bacteria in wildlife populations (Mukerji *et al.*, 2020; Murphy *et al.*, 2021; Rybak *et al.*, 2022).

The presence of virulence factors in *E. coli* strains from wild birds, such as those associated with pathogenicity, such as Shiga toxin-producing *E. coli* and atypical enteropathogenic *E. coli*, further emphasises the potential impact of *E. coli* on the gut microbiome of wild birds and the environment at large (Murphy *et al.*, 2021). In addition, the diversity of *E. coli* phylogroups within individual wild animals and communities reflects the broad distribution and genetic diversity of this bacterium, with implications for biodiversity conservation, agriculture, and public health, especially at the urban-wildland interface (Lagerstrom & Hadly, 2023). Although the focus on *E. coli* pathogenicity and resistance is crucial, it is equally important to consider the environmental and ecological factors that contribute to its spread, highlighting the need for integrated surveillance and intervention strategies.

The genus *Exiguobacterium* is part of the coryneform bacterial group, characterised by aerobic growth, non-spore-forming, irregularly shaped, gram-positive rods (Farrow *et al*., 1994). These bacteria have been found in a variety of habitats ranging from cold to hot environments (Vishnivetskaya *et al*., 2009). Although *Exiguobacterium* strains have been isolated from human clinical samples such as skin, wounds, and cerebrospinal fluid, their clinical relevance is not well understood (Hollis & Weaver, 1981). This genus is more commonly detected in water and soil, suggesting the possibility of sample contamination or environmental presence. The genetic diversity within the genus suggests a wide range of ecological niches, although specific circulating genotypes in South America remain underexplored compared to those in other regions.

The bird populations in Colombia play a crucial role in its ecosystems. According to the IUCN Red List of Threatened Species in 2023, 92 bird species are classified as vulnerable (VU), endangered (EN), or critically endangered (CR). Therefore, monitoring diseases that could impact these species, such as bacterial deseases diseases, is essential (Hollis & Weaver, 1981).

Nevertheless, substantial scientific knowledge gaps remain concerning the distribution of these pathogens and their biological interactions across various regions of Colombia. For instance, Antioquia Province boasts of a rich diversity of bird species and vectors, providing an ideal setting for studying the ecology of these pathogens (Pérez-Rodríguez *et al*., 2014).

There is an urgent need to assess whether the hemobacteria identified in this study pose a threat to the conservation of local bird species. This necessitates comprehensive sampling across a broader range of altitudes, encompassing diverse habitat types and timeframes. It is crucial to estimate the prevalence of these bacteria and other relevant pathogens in critical areas for native birds. This will provide valuable insights into the evolutionary and ecological dynamics of the disease in regions with high host and parasite diversity.

As haemobacterial screening becomes routine in wild bird populations, future research should focus on identifying factors influencing infection and transmission in the area.

DECLARATIONS

Competing interest statement

The authors declare that they have no conflicts of interest.

Ethical considerations

This study was approved by the Ethics and Bioethics Committee for Animal Experimentation of the Universidad Cooperativa de Colombia (Bioethical Concept No. BIO293, Act No. 21-109 of April 28, 2022). In addition, the ANLA granted the Universidad de Antioquia the "Framework permit for the collection of specimens of wild species of biological diversity for noncommercial scientific research purposes" (Resolution 0524 of May 27, 2014, and Resolution 1461 of December 3, 2014), supporting this research approach.

Author contributions

ACF and NMCV had the idea for the article and led the study conception and design. The samples were collected by ACF and AOAP. The molecular analysis of the samples was performed by ILJD, and data analysis by ACF. The literature search and data analysis, as well as the critical revision of the manuscript, were performed by all the authors. The first draft of the manuscript was written by AOAP and NMCV, and all the authors commented on previous versions of the manuscript. All the authors have read and approved the final manuscript.

Data availability statement

All data and materials are available with the corresponding author; the test conditions of the molecular processes are part of the intellectual property of the company TestMol© S.A.S. Dataset of obtained sequences is available at https://doi.org/10.5281/zenodo.14141016

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