Universidad de Caldas

TÍTULO DE LA TESIS:

AVES SILVESTRES Y GARRAPATAS (ACARI: IXODIDAE) Y SU INTERACCIÓN CON *Rickettsia* spp. EN EL DEPARTAMENTO DE ARAUCA, ORINOQUÍA COLOMBIANA.

TESIS QUE PRESENTA **MARELID CARDONA ROMERO**

PARA OBTENER EL GRADO DE MAGÍSTER EN CIENCIAS BIOLÓGICAS

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DIRIGIDA POR **FREDY ARVEY RIVERA PÁEZ, *Ph.D***
CODIRIGIDA POR **GABRIEL JAIME CASTAÑO VILLA, *Ph.D***

Manizales, Caldas, Colombia (mayo, 2021)

Universidad de Caldas

ACTA DE SUSTENTACIÓN DE TESIS

Requisito para la obtención del título de Magister en Ciencias Biológicas

Estudiante: MARELID CARDONA ROMERO **Código: *27117220174***

A las 09:00 a.m. del día 25 de mayo de 2021 se inició el proceso de sustentación de la tesis titulada ***"Aves silvestres y garrapatas (Acari:Ixodidae) y su interacción con Rickettsia spp. en el departamento de Arauca, Orinoquía Colombiana"*** desarrollada por la estudiante **MARELID CARDONA ROMERO,** bajo la dirección de los profesores **Fredy Arvey Rivera,** y codirector **Gabriel Jaime Castaño Villa;** la estudiante fue informada que disponía de una (1) hora para la exposición de su tesis y que posteriormente sería evaluada por los jurados, Mg. **Héctor Jaime Aricapa G. y el Dr. Giovany Guevara Cardona**; sobre la claridad de la exposición y corrección de escritura, originalidad, coherencia y claridad en la argumentación, actualidad y pertinencia de las fuentes.

Posterior a la sustentación de la tesis, fue conferida la siguiente calificación:

Calificación:	4.6	**Recomendación de distinción:**	**Si** ☒	**Tipo de distinción:**	**Meritoria** ☒
			No ☐		**Laureada** ☐

La tesis puede ser distinguida, siempre y cuando la calificación promediada por el grupo de jurados esté entre 4,5 y 4,8 como **Meritoria** o si la calificación promediada está entre 4,9 y 5 como **Laureada**. Del otorgamiento de la distinción realizada por parte de los jurados, se remitirá desde la dirección del programa informe a la Oficina de Admisiones y Registro Académico para que se incluya la constancia en el acta de grado: *Reglamento Estudiantil –Artículo 14, Acuerdo 31 de 2019 del Consejo Académico, que modifica algunos artículos de los capítulos XI y XII del Acuerdo 049 de 2007 del Consejo Académico Universidad de Caldas*.

Con base en lo anterior, el estudiante fue ***Aprobada*** para obtener el título de **Magister en Ciencias Biológicas**. Esta acta está firmada por el director de tesis, los dos jurados evaluadores y por el director del Programa de Maestría en Ciencias Biológicas.

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"*Queda prohibido no sonreír a los problemas, no luchar por lo que quieres, abandonarlo todo por miedo, no convertir en realidad tus sueños*"

Pablo Neruda

DECLARACIÓN DE ORIGINALIDAD

Excepto cuando es explícitamente indicado en el texto, el trabajo de investigación contenido en esta tesis fue efectuado por Marelid Cardona Romero como estudiante de la Maestría en Ciencias Biológicas entre junio de 2018 y diciembre de 2020, bajo la supervisión y orientación de Fredy Arvey Rivera Páez y Gabriel Jaime Castaño Villa.
Las investigaciones reportadas en esta tesis no han sido utilizadas anteriormente para obtener otros grados académicos, ni serán utilizadas para tales fines en el futuro.

Candidato(s):	Nombre	Marelid Cardona Romero
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RESUMEN

Las aves juegan un papel ecológico importante en la distribución y dispersión de garrapatas infectadas a su vez por bacterias gramnegativas del género *Rickettsia* (*Rickettsia* spp.), muchas de las cuáles son patógenas. Las rickettsias se clasifican en cuatro grupos, el grupo tifo, el grupo de las fiebres manchadas, el grupo ancestral y el grupo transicional. En Colombia existen dos regiones endémicas para fiebres manchadas (nororiente de Córdoba y Antioquia y el centro de Cundinamarca). En la región de la Orinoquía se ha detectado la presencia de vectores y rickettsias patógenas, así, como de anticuerpos por serología, que muestran que esta región puede ser endémica para rickettsiosis del grupo de las fiebres manchadas. La Orinoquía es un punto clave de estudio, debido a la presencia de aves migratorias provenientes del hemisferio norte y sur, sin embargo, el papel de las aves silvestres como hospederos de garrapatas infectadas con rickettsias es poco conocido en Colombia, así como su rol de constituir reservorios de rickettsias. El objetivo de la investigación fue el de identificar las garrapatas y rickettsias, asociadas con aves silvestres en la Orinoquía colombiana, así como tratar de comprender posibles interacciones hospedero-vector-patógeno. Se capturaron 606 aves, pertenecientes a 115 especies y 25 familias. Se examinaron un total de 465 garrapatas, pertenecientes a las especies *Amblyomma nodosum, Amblyomma longirostre, Amblyomma mixtum* y *Amblyomma* sp., infestando 15 especies de aves principalmente de las familias Thraupidae, Turdidae y Troglodytidae. Se detectó la presencia de *Rickettsia parkeri* strain Atlantic rainforest en dos garrapatas *A. nodosum,* y se registraron ocho nuevas asociaciones entre aves y garrapatas. Se logró detectar anticuerpos contra rickettsias en 54 especies de aves residentes y migratorias (boreales y australes) en los municipios de Arauca, Tame y Cravo Norte (Arauca) y se detectó en sangre *Rickettsia* sp. únicamente en un individuo de la especie *Phacellodomus rufifrons* (Castillero Llanero). Estos resultados evidencian la importancia de las aves silvestres como hospederos de garrapatas infectadas por rickettsias patógenas y el contacto previo de estas aves con garrapatas portadoras de rickettsias en la región de la Orinoquía, colombiana.

Palabras clave: Hospedero, infección, parasitismo, patógeno, reservorio.

ABSTRACT

Birds play an important ecological role in the distribution and dispersal of ticks infected in turn by gram-negative bacteria of the genus *Rickettsia* (*Rickettsia* spp.), many of which are pathogenic. Rickettsiae are classified into four groups, the typhus group, the spotted fever group, the ancestral group, and the transitional group. In Colombia there are two endemic regions for spotted fevers (northeast of Córdoba and Antioquia and central Cundinamarca). In the Orinoquía region, the presence of vectors and pathogenic rickettsiae has been detected, as well as antibodies by serology, which show that this region may be endemic for rickettsiosis of the group of spotted fevers. The Orinoquía is a key point of study, due to the presence of migratory birds from the northern and southern hemispheres, however, the role of wild birds as hosts of ticks infected with rickettsiae is little known in Colombia, as well as their role in constitute reservoirs of rickettsiae. The objective of the research was to identify ticks and rickettsiae, associated with wild birds in the Colombian Orinoquía, as well as to try to understand possible host-vector-pathogen interactions. 606 birds were captured, belonging to 115 species and 25 families. A total of 465 ticks were examined, belonging to the species *Amblyomma nodosum*, *Amblyomma longirostre, Amblyomma mixtum* and *Amblyomma* sp., infesting 15 species of birds, mainly from the families Thraupidae, Turdidae and Troglodytidae. The presence of *Rickettsia parkeri* strain Atlantic rainforest was detected in two *A. nodosum* ticks, and eight new associations between birds and ticks were recorded. Antibodies against rickettsiae were detected in 54 species of resident and migratory birds (boreal and southern) in the municipalities of Arauca, Tame and Cravo Norte (Arauca) and *Rickettsia* sp. only in one individual of the species *Phacellodomus rufifrons* (Castillero Llanero). These results show the importance of wild birds as hosts for ticks infected by pathogenic rickettsiae and the previous contact of these birds with rickettsia-bearing ticks in the Orinoquía region of Colombia.

Keywords: Host, infection, parasitism, pathogen, reservoir.

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1. CAPÍTULO INTRODUCTORIO

Las aves silvestres cumplen un papel ecológico importante en la dispersión y en el ciclo de vida de algunas especies de garrapatas, al hospedar principalmente estadios inmaduros como larvas y ninfas, y en algunos casos, estadios adultos (Ogrzewalska et al., 2009a; Flores et al., 2014; Lugarini et al., 2015; Ramos et al., 2015). Las aves hospederas de garrapatas, a su vez, favorecen la dispersión y transporte de patógenos como virus, bacterias y protozoos asociados con estos ectoparásitos (Mukherjee et al., 2014; Cohen et al., 2015; Budachetri et al., 2017; Luz et al., 2017).

Las especies de garrapatas que mayoritariamente parasitan aves involucradas en la transmisión de patógenos, son generalmente garrapatas duras de la familia Ixodidae (Pacheco et al., 2012; Parola et al., 2013), pertenecientes principalmente a los géneros *Amblyomma, Ixodes* y *Haemaphysalis* (Barros-Battesti et al., 2006). Las garrapatas duras durante la ingesta de alimento, se adhieren por un largo período a su hospedero en el que pueden transmitir y adquirir patógenos, entre los cuales se encuentran las bacterias del género *Rickettsia* (Mukherjee et al., 2013; Pfäffle et al., 2013; Oteo et al., 2014; Luz et al., 2017). Las garrapatas infectadas pueden proporcionar una fuente de infección para otros ectoparásitos y hospederos (Hornok et al., 2014).

Las rickettsias son bacterias Gramnegativas intracelulares obligadas, que se pueden encontrar libres en el citoplasma de las células o dentro del núcleo celular de sus hospederos vertebrados (Quintero-Vélez et al., 2012). Las bacterias del género *Rickettsia* se agrupan principalmente en cuatro grupos, i) el grupo tifo (TG, por sus siglas en inglés) con dos miembros, *Rickettsia prowazekii* y *Rickettsia typhi,* ii) el grupo de las fiebres manchadas (SFG, por sus siglas en inglés), que incluye más de 20 especies, entre ellas *Rickettsia rickettsii* y *Rickettsia parkeri*, iii) el grupo transicional (TRG, por sus siglas en inglés) del cual hacen parte *Rickettsia akari, Rickettsia felis*; y iv) el grupo ancestral (AG, por sus siglas en inglés) con los miembros *Rickettsia bellii* y *Rickettsia canadensis* (Quintero-Vélez et al., 2012; Hidalgo et al., 2013). Los grupos TG y SFG son los de mayor preocupación para la salud pública, debido a la patogenicidad de sus rickettsias

(Londoño et al., 2017). Las rickettsias del SFG son causantes de enfermedades conocidas en el continente americano como Fiebre Manchada de las Montañas Rocosas (Estados Unidos), Fiebre Maculosa Brasileña (Brasil) y Fiebre de Tobia (Colombia), estas son causadas por el agente etiológico *Rickttsia rickettsii* con tasas de letalidad entre el 20 y 95% (Abarca y Oteo et al., 2014). A lo largo del continente americano también existen dos cepas, *Rickettsia parkeri* sensu stricto y *Rickettsia parkeri* strain Atlantic rainfoest causantes de una variante menos patógena y no letal de la Fiebre de las Montañas Rocosas (Londoño et al., 2019).

Actualmente, en el mundo se conocen aproximadamente 955 especies de garrapatas, perteneciente a las familias: Ixodidae (736 especies), Argasidae (218 especies), Nuttalliellidae (1 especie) (Barros-Battesti et al., 2006; Guglielmone et al., 2014; Nava et al., 2017; Dantas-Torres et al., 2019). La familia Ixodidae comprende 736 especies de garrapatas distribuidas en las regiones Tropical y Neotropical del mundo (Bowman et al., 2003; Barros-Battesti et al., 2006; Hornok et al., 2016; Labruna et al., 2016; Muñoz-Leal et al., 2016; Apanaskevich and Bermúdez, 2017; Martins et al., 2019).

En Colombia, se reportan 43 especies de garrapatas de la familia Ixodidae de los géneros *Amblyomma, Ixodes, Haemaphysalis, Rhipicephalus* y *Dermacentor* (Guglielmone et al., 2003; Rivera-Paéz et al., 2018a; Ortíz-Giraldo et al., 2020), donde, los géneros *Amblyomma, Rhipicephalus* y *Dermacentor*, están fuertemente relacionadas con la transmisión de rickettsias del grupo de las fiebres manchadas (Hidalgo et al., 2013). El primer brote de rickettsiosis por rickettsias del grupo de las fiebres manchada en Colombia ocurrió entre 1934 y 1936 en Tobia, Cundinamarca, el cual fue reportado por el Doctor Luis Patiño como un brote producido por bacterias compatibles con *Rickettsia* sp. (Quintero-Vélez et al., 2012; Hidalgo et al., 2013). Tras décadas de silencio epidemiológico, en el municipio de Villeta, Cundinamarca, se detectaron anticuerpos contra rickettsias en humanos y se estableció una seroprevalencia del 40.2% (Quintero-Vélez et al., 2012). En diferentes municipios del noroeste de Colombia como Necoclí y Turbo (Antioquia) y Los Córdobas (Córdoba), entre 2006 y 2008 se presentaron diferentes brotes de rickettsiosis causados por *R. rickettsii* con una letalidad entre el 26 y

54% (Acosta et al., 2006; Hidalgo et al., 2011; Pacheco et al., 2008). Otros reportes importantes, fueron la detección de *R. rickettsii* y *Rickettsia amblyommii* en *Amblyomma patinoi* en el departamento de Cundinamarca (Faccini-Martínez et al., 2015; 2016) y la detección de *Rickettsia* sp. strain Atlantic rainforest en *Amblyomma ovale* en los departamentos de Antioquia y Córdoba (Londoño et al., 2014).

Debido a los brotes causados por rickettsias patógenas en la región noroeste de Córdoba y Antioquía, y en el centro del país en Cundinamarca, estas dos zonas fueron definidas como regiones endémicas para fiebres manchadas. De igual manera, se ha propuesto considerar como tercera región endémica para fiebres manchadas a la Orinoquía colombiana (Rivera-Páez et al., 2018b). Esto debido a evidencias como seropositividad en humanos y animales domésticos frente a rickettsias y a la detección molecular de *R. rickettsii* y su vector *Amblyomma mixtum* en esta región (Miranda et al., 2011; Riveros-Pinilla et al., 2015; Gómez-Quintero et al., 2017; Rivera-Páez et al., 2016, 2018a, 2018b). Es así, que teniendo en cuenta la importancia de las garrapatas y rickettsias para la salud pública, la Orinoquía colombiana, se puede considerar como una región clave para el estudio de las asociaciones entre aves silvestres, garrapatas y rickettsias, maxime si se tiene en cuenta que esta región es un punto de encuentro de aves migratorias provenientes de los hemisferios Norte y Sur (McNish, 2007; Ocampo-Peñuela, 2010).

Diferentes estudios sobre asociaciones entre aves, garrapatas y rickettsias se han realizado a través de toda América (Ogrzewalska et al., 2012; Mukherjee et al., 2014; Ogrzewalska et al., 2014; 2015; Cohen et al., 2015; Novakova et al., 2015; Flores et al., 2016; Ogrzewalska et al., 2016; Bermúdez et al., 2020). En Colombia, el país con la diversidad de aves más alta del mundo entre especies residentes y migratorias (Avendaño et al., 2017), los estudios sobre la infestación de aves por garrapatas son escasos (Osorno-Mesa, 1940; González-Acuña et al., 2005; Martínez-Sánchez et al., 2020a). De manera similar, los estudios sobre la detección de rickettsias en garrapatas que parasitan aves en Colombia se limitan al estudio realizado por Martínez-Sánchez et al. (2020b), en el departamento de Caldas. Adicionalmente, las investigaciones focalizadas en la detección de rickettsias en aves, se restringen a estudios en algunas naciones europeas y africanas, en Estados

Unidos y Brasil (Lundgren et al., 1966; Kelly et al., 1996; Ioannou et al., 2009; Stańczak et al., 2009; Maciel et al., 2013, 2016; Hornok et al., 2014; Mukherjee et al., 2014; Cohen et al., 2015; Berthová et al., 2016; Budachetri et al., 2016; Erwin et al., 2016; Ebani et al., 2017).

Teniendo en cuenta la importancia de las aves silvestres como hospederas de garrapatas infectadas por rickettsias pertenecientes al grupo de las fiebres manchadas (SFG) como *R. rickettsii*, *R. parkeri*, *Rickettsia amblyommatis* y *Rickettsia rhipicephali* (Sonenshine and Clifford, 1973; Lugarini et al., 2015; Zeringóta et al., 2017). Así como el desconocimento actual en Colombia del rol de las aves silvestres como reservorios de rickettsias (Ogrzewlaska et al., 2008; Pacheco et al., 2012; Ogrzewlaska and Pinter, 2016), los objetivos de la presente investigación fueron identificar las especies de garrapatas asociadas con las aves silvestres y detectar la posible infección por rickettsias en garrapatas. De igual manera, se planteó detectar e identificar rickettsias en aves silvestres que podrían estar actuando como reservorios en el departamento de Arauca, Orinoquía colombiana.

2. MATERIALES Y MÉTODOS

2.1. Área de studio

El estudio se realizó en el departamento de Arauca, ubicado al Oriente de Colombia en la región de la Orinoquía, en los municipios de Arauca, Cravo Norte y Tame. Los ecosistemas de la región hacen parte de la llanura baja, conformados por sabanas inundables, esteros, bosques de galería y bosques aislados conocidos localmente como "*matas de monte*" (McNish, 2007). La región presenta un clima típico de sabana, con una estación lluviosa bien definida entre junio y julio y una estación de intensa sequía entre diciembre y abril. Este patrón hace que algunas veces las sabanas de la Orinoquía entren en períodos de inundación y de sequía durante estas estaciones, respectivamente. Esta región presenta una temperatura alta todo el año, con un rango de temperatura entre 22° y 27°C (McNish, 2007).

Las localidades muestreadas se pueden diferenciar en dos subregiones, la Sabana Inundable y el Piedemonte Llanero (Rodríguez-Durán, 2019). El Piedemonte Llanero constituye una subregión contigua a la Cordillera Oriental de Colombia, entre los 250 m y 500 m de altitud. Ambas subregiones se destinan para actividades agropecuarias como cultivos de arroz, palma de aceite, maíz y plátano y la ganadería extensiva (Viloria de la Hoz, 2009).

Inicialmente se muestreó la avifauna para la toma de muestras de garrapatas y sangre, entre noviembre y diciembre de 2018 en la subregión de la Sabana Inundable en el municipio de Arauca en cuatro localidades de la vereda Las Plumas y en una localidad del municipio de Cravo Norte. Durante el mes de marzo de 2019 se tomaron muestras en el municipio de Tame localizado en el Pie de Monte Llanero, en la vereda Santa Inés. Por último, entre agosto y julio de 2019, se tomaron muestras en el municipio de Arauca y en la vereda El Socorro. Las fechas de los muestreos de la avifauna concordaron con el arribo de las aves migratorias provenientes del hemisferio norte (migratorias boreales) y del hemisferio sur (migratorias australes).

2.2. Captura de aves y garrapatas

Para la captura de aves se utilizaron ocho redes de niebla (12 m largo x 2 m ancho, 36 mm ojo de malla) en cada localidad de muestreo. Las redes de niebla fueron dispuestas aleatoriamente y abiertas entre las 6:00 y 17:00 h. Las aves fueron identificadas taxonómicamente de acuerdo con Remsen et al. (2020). El estatus de residencia de las aves (i.e., residente, migratoria boreal o austral) se definió de acuerdo con Avendaño et al. (2017). Cada ave se examinó buscando garrapatas en todo el cuerpo durante 5 min y posteriormente cada una fue marcada mediante un pequeño corte en la primera rectriz de la cola, para evitar el reconteo de individuos examinados. Todas las aves fueron liberadas en el sitio de captura. Las garrapatas encontradas fueron extraídas con pinzas entomológicas y conservadas en tubos eppendorf de 1.5ml con etanol al 96%.

2.3. Obtención de muestras de sangre

Se realizó por venopunción de la vena braquial y fueron colectadas en tubos de microhematocrito de 60 µL sin anticoagulante (Maciel et al., 2013). De cada muestra de sangre se depositaron aproximadamente 40 µL en tarjetas FTA (Whatman Ltda., Inglaterra) para conservar el ADN y utilizarlo en posteriores pruebas. El volumen de sangre restante se centrifugó para la obtención de suero. Las muestras obtenidas de sangre y suero se conservaron en los laboratorios de Genética y del grupo de Investigación Biosalud de la Universidad de Caldas.

Los procedimientos de colecta en campo y la obtención de muestras contó con la aprobación del Comité de Bioética de la Facultad de Ciencias Exactas y Naturales de la Universidad de Caldas y se realizó bajo el "Permiso Marco otorgado a la Universidad de Caldas por la Autoridad Nacional de Licencias Ambientales (ANLA) de Colombia (Resolución 02497 de diciembre 31 de 2018)".

2.4. Identificación de garrapatas y rickettsias

Las garrapatas colectadas se identificaron taxonómicamente por morfología externa utilizando literatura y claves taxonómicas (Kohls, 1956; Jones et al., 1972; Barros-Battesti et al., 2006; Mehlhorn, 2008; Martins et al., 2010; Nava et al., 2014; 2017). La

prevalencia de infestación por garrapatas en aves se calculó como: PIG = (*Número de individuos infestados / Número de individuos examinados*) *x 100*.

Las garrapatas (ninfas y larvas) se confirmaron molecularmente, realizando una extracción de ADN utilizando los kits *DNeasy Blood and Tissue de Qiagen y Wizard® Genomic DNA Purification* de Promega, siguiendo el protocolo sugerido de los fabricantes. Se realizó la PCR de dos genes mitocondriales: el gen 16S rDNA amplificando un fragmento aproximado de 460pb con los iniciadores 16S F 5'-CCGGTCTGAACTCAGATCAAGT-3', 16S R 5'-CTGCTCAATGATTTTTTAAATTGCTGTGG-3' (Norris et al., 1996; Mangold et al., 1998) y un fragmento de 700pb del citocromo oxidasa mitocondrial subunidad I gen COI, usando los iniciadores LCO1490 F 5'-GGTCAACAAATCATAAAGATATTGG-3' HCO2198 R 5'-TAAACTTCAGGTGACCAAAAAATCA-3' (Folmer et al., 1994). Los vouchers de las garrapatas recolectadas se depositaron en la Colección de Ectoparásitos del Museo De Historia Natural de la Universidad de Caldas (MHN-UCa).

Para la detección e identificación molecular de especies de *Rickettsia* se amplificó un fragmento aproximado de 401pb del gen citrato sintasa gltA con los iniciadores CS-78 y CS-323 (Labruna et al., 2004), este gen está presente en todas las especies del género *Rickettsia* (Figura 1). Las muestras positivas para gltA, se usaron para una segunda PCR con los iniciadores rompB-OF y rompB-OR, propuestos por Choi et al. (2005), que amplifican un fragmento de 511pb de la proteína de la membrana externa ompB, presente en las especies de rickettsias del grupo de las fiebres machadas (SFG) (Choi et al., 2005). En cada reacción se utilizó un control negativo (agua ultrapura) y un control positivo (*Rickettsia vini*) suministrado por el Dr. Marcelo Bahia Labruna del *Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo (USP-Brasil).*

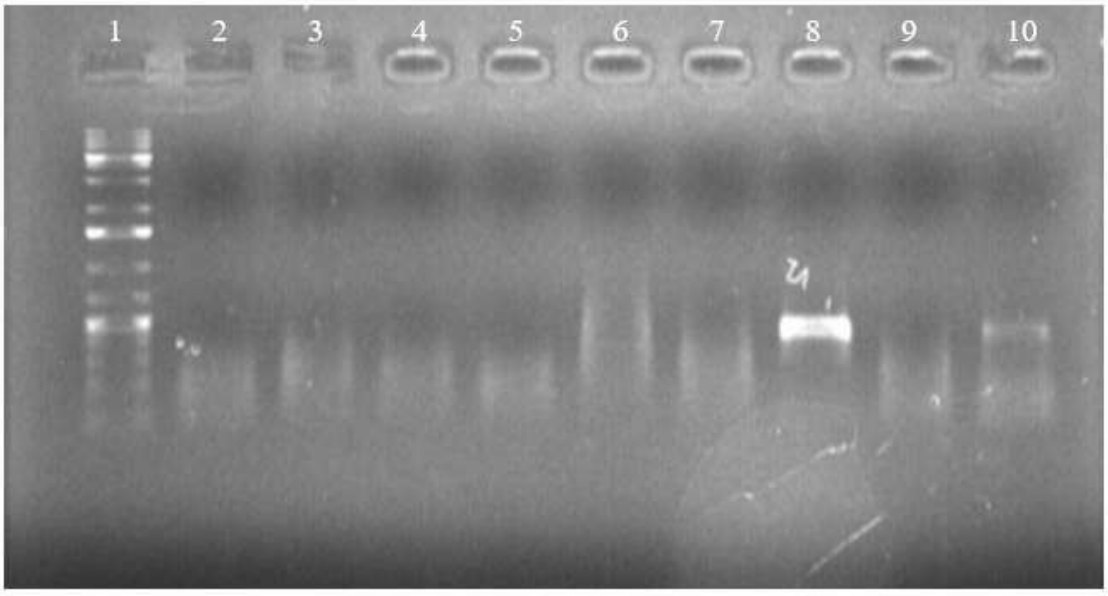

Figura 1. Amplificación por PCR del gen citrato sintasa (*gltA*). Canal 1: marcador de peso molecular 1kb. Canal 2: control negative (agua ultrapure). Canal 3-7, 9: muestras de aves negativas para rickettsias. Canal 8: muestra de *P. rufifrons* positiva para *Rickettsia.* Canal 10: Control positivo (*R. vini*).

Los productos de PCR se visualizaron por electroforesis horizontal en geles de agarosa al 1% con tampón de corrida TBE 1X (pH 8.0) a 110V/50mA y coloreados con *SYBR® Safe.* Los productos se visualizaron en un foto documentador Gel Doc-It2 310 (UVP) y se purificaron utilizando el kit *Wizard® SV Gel and PCR Clean-Up System* de Promega, siguiendo el protocolo del fabricante. El servicio de secuenciación de ADN lo realizó *Macrogen Inc* (Corea del Sur), y las secuencias ([MT471971-MT471980], [MT439632], [MT501330], [MT501331], [MT501328], [MT501328]) se depositaron en el *GenBank.* El análisis de la calidad de las secuencias de ADN, se realizó en el programa *Geneious Prime®* 2019.1.3. Los alineamientos y análisis de las secuencias se realizaron en el [programa Mega versión X (Kumar et al., 2018). La identificación y análisis de especies de garrapatas y rickettsias tuvo en cuenta las estimativas de similitud con las secuencias públicas del *GenBank* y del *BOLD* (*Barcode of Life Data Systems*, www.barcodinglife.com).

2.5. Detección de anticuerpos frente a rickettsias

El suero de cada ave se analizó por ensayos de Inmunofluorescencia indirecta (IFA por sus siglas en inglés) siguiendo la metodología propuesta por Horta et al. (2004). Una

dilución 1:64 de la muestra de suero de cada ave se colocó en contacto con células Vero infectadas con *R. rickettsii, R. parkeri, R. amblyommatis, R. rhipicephali* y *R. bellii*, fijadas en láminas para Inmunofluorescencia indirecta. Estas láminas fueron suministradas por el Doctor Marcelo B. Labruna del *Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo (USP-Brasil)*. La formación para el manejo de la técnica de Inmunofluorescencia indirecta se realizó en el Laboratório de *Doenças Parasitárias do Departamento de Medicina Veterinária Preventiva e Saúde Animal da Universidade de São Paulo (USP-Brasil)* durante el período de pasantía realizada por la autora del presente trabajo de grado, entre 25/09/2019 al 21/10/2019.

Para la detección de anticuerpos contra rickettsias se utilizó el conjugado con fluoresceína IgY *Anti-Chicken* producido en cabra (*Jackson ImmunoResearch Laboratories*) en una dilución 1:400 y se visualizó con un microscopio de Luz ultravioleta (Motic BA410E) a un aumento de 400X en el laboratorio del grupo de Investigación Biosalud de la Universidad de Caldas y en el *Laboratório de Doenças Parasitárias do Departamento de Medicina Veterinária Preventiva e Saúde Animal da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo.* En cada lámina portaobjetos se analizó un suero previamente establecido como no reactivo (control negativo) y un suero reactivo (control positivo) (Figura 2). Cuando las muestras de suero fueron reactivas para al menos uno de los cinco antígenos en la dilución inicial, se consideraron positivas y se procedió con las siguientes diluciones para la titulación de las muestras hasta la dilución 1:1024. Todas las muestras con títulos al menos cuatro veces mayores para una especie de *Rickettsia* en comparación con las otras, se consideraron positivas para dicha especie (Horta et al., 2004; Pacheco et al., 2007).

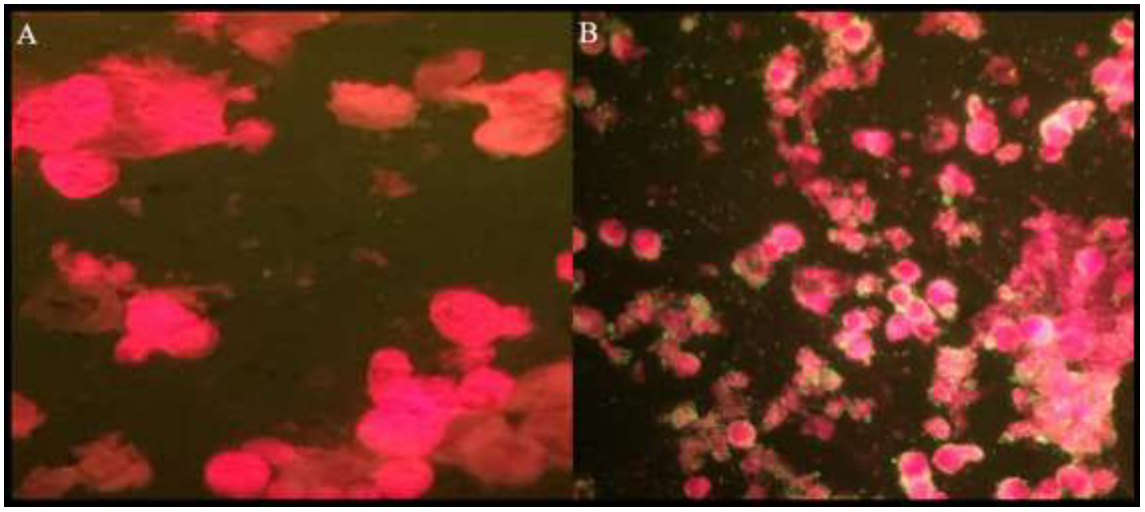

Figura 2. Ensayo de Inmunofluorescencia indirecta (IFA) en aves silvestres a una dilución 1:64. (A) Muestra utilizada como control negativo. (B) Muestra utilizada como control positivo.

3. RESULTADOS

Los resultados se presentan a continuación en dos capítulos. Cada capítulo consta de un artículo científico, publicado, sometido o en preparación para ser sometido en una revista internacional especializada en el área estudio.

3.1. CAPÍTULO I

Status: Publicado, 12 de septiembre de 2020

Autores: **Marelid Cardona-Romero**, Estefani T. Martínez-Sánchez, Johnathan Alvarez Londoño, William D. Tobón-Escobar, Paula A. Ossa-López, Jorge E. Pérez-Cárdenas, Héctor E. Ramírez-Chaves, Giovanny Blandón-Marín, Ludwin A. Cuervo, Gabriel J. Castaño-Villa, Fredy A. Rivera-Páez.

Título del artículo: *Rickettsia parkeri* strain Atlantic rainforest in ticks (Acari: Ixodidae) of wild birds in Arauca, Orinoquia region of Colombia

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3.2. CAPÍTULO II

Status: En preparación

Autores: **Marelid Cardona-Romero**, Estefani T. Martínez-Sánchez, Johnathan Alvarez-Londoño, Jorge E. Pérez-Cárdenas, Paula A. Ossa-López, Gabriel J. Castaño Villa, Lina C. Binder, Álvaro A. Faccini-Marínez, Fredy A. Rivera-Páez

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Revista proyectada: Veterinary Parasitology: Regional Studies and Reports.

3.1. CAPÍTULO I

Rickettsia parkeri strain Atlantic rainforest in ticks (Acari: Ixodidae) of wild birds in Arauca, Orinoquia region of Colombia

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Rickettsia parkeri strain Atlantic rainforest in ticks (Acari: Ixodidae) of wild birds in Arauca, Orinoquia region of Colombia

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ABSTRACT

Birds are important hosts for the development of the immature stages of several tick species that are vectors for disease-causing microorganisms in animals and humans. Colombia has the highest number of bird species worldwide; however, there is scarce data on the role of birds in the circulation of ticks and their associated pathogens, such as rickettsiae. The department of Arauca has a high diversity of resident and migratory (boreal and austral) birds and ticks associated with the transmission of *Rickettsia*. The objective of this research was to identify tick species parasitizing birds and to detect *Rickettsia* species in these ectoparasites. We conducted samplings in the municipalities of Arauca, Cravo Norte, and Tame between November of 2018 and August of 2019. Birds were captured using mist nets and examined for the presence of tick species. The collected ticks were morphologically and molecularly identified. Furthermore, we detected rickettsiae in ticks by amplifying fragments of the citrate synthase (*gltA*) and outer membrane protein (*ompB)* genes. We captured 606 birds belonging to 25 families and 115 species. Tick infestation rate was 3.3% (20/606) in the birds captured and eight new associations between wild birds and ticks are reported for the American continent. We identified four tick species: *Amblyomma nodosum, Amblyomma longirostre*, *Amblyomma mixtum*, and *Amblyomma* sp.. Moreover, we confirmed the presence of *Rickettsia parkeri* strain Atlantic rainforest in *A. nodosum*, a medically-relevant rickettsia due to cases of rickettsiosis in the American continent. This finding manifests the importance of wild birds as hosts and dispersal agents of ticks infected with pathogenic rickettsiae, as well as the need to monitor migratory birds in the Orinoquia and other regions of Colombia and America.

1. Introduction

Wild birds have an important role in the life cycle of several tick species since birds serve as hosts for the immature stages of larvae and nymphs and, in some cases of ornithophilic ticks (Ogrzewalska et al., 2009a; Flores et al., 2014; Ramos et al., 2015). Ticks can act as vectors or reservoirs of pathogenic bacteria, such as rickettsiae, which can be transmitted to animals and humans (Sonenshine et al., 2002; Parola

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et al., 2013; Luz et al., 2017). Ticks have limited locomotion capacity; therefore, their dispersal largely depends on hosts (Randolph, 1998). In this sense, bird hosts can disperse ticks between habitats or even continents during migrations (Baneth, 2014; Mukherjee et al., 2014; Cohen et al., 2015; Budachetri et al., 2017). In America, there are records of several ticks species of the genera *Amblyomma, Ixodes, Haemaphysalis* (family Ixodidae), *Ornithodoros*, and *Argas* (family Argasidae) that parasitize resident and migratory birds (Barros-Battesti et al., 2006; Ogrzewalska et al., 2008, 2015; Mukherjee et al., 2014; Cohen et al., 2015). Colombia has the highest bird diversity in the world, with 1632 resident species and 139 migratory boreal and austral species (Avendaño et al., 2017). In Colombia, there are 58 tick species (43 Ixodidae and 15 Argasidae) (Rivera-Páez et al., 2018a), including eight species associated with wild birds: *Ixodes auritulus* (González-Acuña et al., 2005), *Amblyomma calcaratum, Amblyomma dissimile, Amblyomma longirostre, Amblyomma nodosum, Amblyomma ovale, Amblyomma varium, and Haemaphysalis leporispalustris* (Osorno-Mesa, 1940; Martínez-Sánchez et al., 2020). The record of *Ixodes brunneus* in *Calochaetes coccineus* should be revised (Osorno-Mesa, 1940). In addition, three *Ixodes* species, which yielded low identity DNA sequences (≤95%) to any tick species in GenBank are also known (Martínez-Sánchez et al., 2020).

Ticks can transmit bacteria that cause rickettsial diseases (Sonenshine and Clifford, 1973; Ogrzewalska et al., 2009b; Cohen et al., 2015). In particular, these diseases are attributed to infection by Gram-negative bacteria of the genus *Rickettsia,* which comprise four groups: i) the typhus group (TG) with two members, namely *Rickettsia prowazekii* and *Rickettsia typhi*; ii) the spotted fever group (SFG) that contains more than 20 species, including *Rickettsia rickettsii* and *Rickettsia parkeri*; iii) the transitional group (TRG), which comprises *Rickettsia akari* and *Rickettsia felis*; and iv) the ancestral group (AG) that includes *Rickettsia belli* and *Rickettsia canadensis* (Quintero et al., 2013). Specifically, TG and SFG generate the highest level of concern for public health (Londoño et al., 2017); for instance, *R. rickettsii* is the most pathogenic species in this genus, with reported lethality rates between 20% and 95% for the American continent (Abarca and Oteo, 2014). Particularly, in Colombia, there are lethality rates between 26.6% and 95% (Quintero et al., 2013; Miranda et al., 2017). *R. rickettsii* is the causal agent of Rocky Mountain spotted fever (RMSF), Brazilian spotted fever, or Tobia fever in Colombia (Labruna et al., 2011; Oteo et al., 2014). Moreover, there are two strains of *R. parkeri* known to cause rickettsiosis, namely *R. parkeri* sensu stricto and *R. parkeri* strain Atlantic rainforest (Londoño et al., 2019). Like in other spotted fever group (SFG) rickettsiae, *R. parkeri* is transovarially (from female to eggs) and transestadially (from one life history stage to the next) transmitted to ticks. Also, *R. parkeri* can be horizontal acquired while tick feeds on a rickettsemic host (Goddard, 2003; Walker and Ismail, 2008).

Historically, *R. rickettsii* has caused outbreaks of febrile illness in several regions of Colombia, such as the central (department of Cundinamarca) and northwestern regions (departments of Cordoba and Antioquia); therefore, these two regions of considered endemic areas for rickettsiosis (Patiño et al., 1937; Patiño, 1941; Acosta et al., 2006; Hidalgo et al., 2007a, Hidalgo et al., 2007b, 2011; Vélez et al., 2012). Additionally, the Orinoquia region has also been proposed as an endemic area for rickettsial disease associated with the SFG, due to the confirmed circulation of rickettsiae and their vector *Amblyomma mixtum* in this region (Miranda et al., 2011; Riveros-Pinilla et al., 2015; Rivera-Páez et al., 2016; Gómez-Quintero et al., 2017; Rivera-Páez et al., 2018b). In the last decade, there were reports of the pathogenic strain *Rickettsia parkeri* Atlantic rainforest associated with *Amblyomma ovale* in the departments of Cordoba and Antioquia (northwestern region).

R. parkeri strain Atlantic rainforest is phylogenetically closely related to *R. parkeri*, *Rickettsia africae*, and *Rickettsia sibirica* (Spolidorio et al., 2010; Londoño et al., 2014; Nieri-Bastos et al., 2018). *R. africae* and *R. sibirica* are pathogenic species distributed in the Old World, and the symptoms they cause are similar to those caused by New World species such as *R. parkeri* sensu stricto, and *R. parkeri* strain Atlantic rainforest (Paddock et al., 2004; Parola et al., 2005; Pacheco et al., 2012). Using genetic evidence, Nieri-Bastos et al. (2018), defined that Atlantic rainforest is *R. parkeri* strain found in the southern part of South America that is transmitted by ticks of the *A. ovale* complex (i.e., *A. ovale* and *Amblyomma aureolatum*). Other tick species of the *Amblyomma maculatum* complex (*A. maculatum, Amblyomma triste*, and *Amblyomma tigrinum*), *A. nodosum*, *Amblyomma parvitarsum* and *Dermacentor parumapertus* have been involved in the transmission of *R. parkeri* in America (Paddock et al., 2004, 2017; Nieri-Bastos et al., 2018; Londoño et al., 2019).

A. ovale was found parasitizing resident and migratory birds (e.g., *Formicivora grisea* and *Parkesia noveboracensis*) in Colombia (Martínez-Sánchez et al., 2020). However, *R. parkeri* strain Atlantic rainforest has not been detected in ticks associated with birds, although it has been detected in ticks on wild and domestic mammals in the northwestern region of Colombia (Londoño et al., 2014, 2017). The only reports of genus *Rickettsia* associated with ticks in the Orinoquia region, are the reports of *R. rickettsii* on ticks of domestic animals (Rivera-Páez et al., 2018b).

Given that migratory boreal and austral birds converge in the Colombian Orinoquia region (McNish, 2007; Ocampo-Peñuela, 2010), these bird species could serve as hosts and dispersal agents of ticks infected with rickettsiae. The department of Arauca in the Orinoquia (Eastern Plains) is an ideal site to study the associations among birds, ticks, and rickettsiae. Given the above, this study aimed to identify tick species associated with wild birds and detect ticks infected with rickettsiae.

2. Materials and methods

2.1. Study area

This study was conducted in eight localities in the municipalities of Arauca, Cravo Norte, and Tame, located in the department of Arauca in the Colombian Orinoquia (Table 1, Fig. 1). The region shows a typical savanna climate with a well-defined wet season between June and July and a very dry season between December and April. This precipitation pattern leads to occasional periods of flooding and drought in the Orinoquia savanna during the corresponding seasons. This region also shows high temperatures year-round with a mean of 27 °C (McNish, 2007).

The sampled localities are divided into two sub-regions: the flooded savanna (*Sabana Inundable*) and the Llanos foothills (*Piedemonte Llanero*) (Rodríguez-Durán, 2019). The flooded savanna comprises estuaries, gallery forests, and isolated forests locally known as “matas de monte” (McNish, 2007). The Llanos foothills are a sub-region between 250 m and 500 m of elevation, adjacent to the Eastern mountain range of Colombia. Both sub-regions are destined for agropecuary activities, such as rice, oil palm, corn, and plantain crops and extensive livestock farming (Viloria de la Hoz, 2009). We conducted samplings in the eight localities to identify bird species parasitized by ticks and to detect possible infections of these ticks with rickettsiae. Localities 1–5 were sampled between November and December of 2018, locality 6 was sampled in March, and localities 7 and 8 were sampled between July and August of 2019 (Table 1, Fig. 1). The sampling dates coincided with the arrival of migratory birds from the northern (migratory boreal) and southern (austral migratory) hemispheres.

2.2. Bird captures and tick collection

Birds were captured using eight mist nets at each locality (12 m long × 2 m wide, 36 mm mesh). The mist nets were randomly installed and opened between 6:00 and 17:00 h. The birds were taxonomically classified according to Remsen et al. (2020). The residency status of the birds (i.e., resident, migratory boreal or austral) was defined based on Avendaño et al. (2017). Each bird was completely examined for ticks for

Table 1
Descriptions of the localities sampled in the department of Arauca, Colombia, where birds were captured in this study.

Municipality	Locality	Locality number[a]	Geographical coordinates	Altitude (m above sea level)	Habitat type
Arauca	Las Plumas	1	06°36′40″ N 70°31′51″ W	120	Floodplain forest, Gallery forest
Arauca	Las Plumas	2	06°37′01″ N 70°31′30″ W	123	Floodplain forest, Medano
Arauca	Las Plumas	3	06°36′15″ N 70°29′52″ W	123	Floodplain forest
Arauca	Las Plumas	4	06°36′15″ N 70°29′52″ W	112	Floodplain forest
Cravo Norte	El Deleite	5	06°32′15″ N 70°31′14″ W	111	Floodplain forest
Tame	Santa Inés	6	06°24′52″ N 71°32′04″ W	253	Agricultural area
Arauca	Km 9, via Arauca-Arauquita	7	07°00′53″ N 70°44′36″ W	120	Wooded areas; High stubble
Arauca	El Socorro	8	06°46′39″ N 70°42′25″ W	134	Floodplain forest, Gallery forest

[a] Locality numbers indicated in Fig. 1.

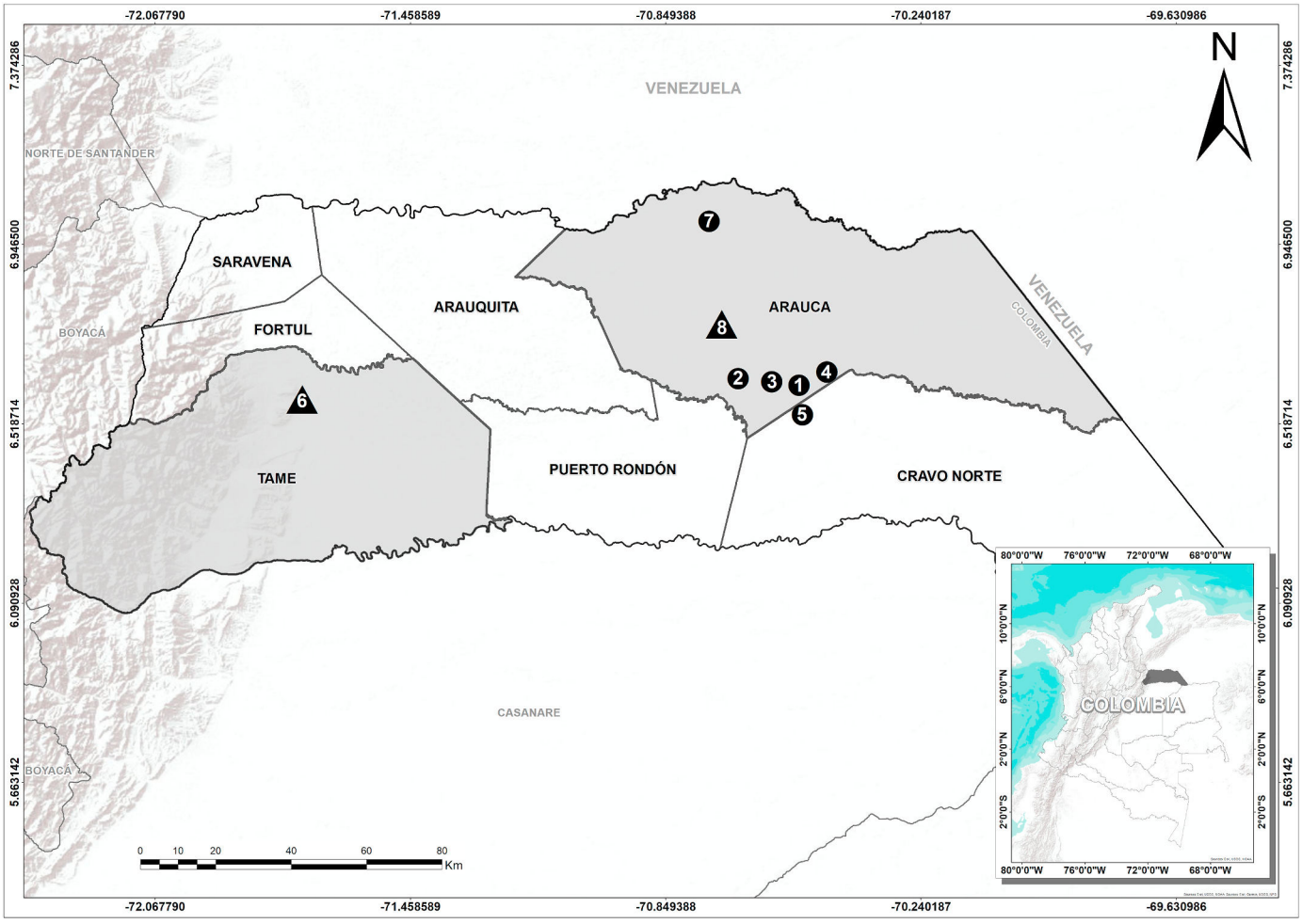

Fig. 1. Localities sampled in the municipalities of Arauca, Cravo Norte, and Tame and reports of *Rickettsia* spp. in the study area (▲*Rickettsia parkeri* strain Atlantic rainforest).

5 min. The ticks were collected with entomological tweezers and conserved in Eppendorf tubes with 96% ethanol. The birds were marked with a small cut in the first rectrix of the tail to avoid re-counting the individuals and then, released at the capture site.

2.3. Identification of ticks and rickettsiae

The ticks collected were taxonomically identified based on their external morphology according to the literature and taxonomical keys (Kohls, 1956; Jones et al., 1972; Barros-Battesti et al., 2006; Mehlhorn, 2008; Martins et al., 2010; Nava et al., 2014, 2017). The prevalence of tick infestation in birds was calculated as *(Number of infested individuals/Number of examined individuals) x 100*.

The nymphs and larvae were molecularly confirmed through PCR amplification of two mitochondrial gene fragments. First, DNA extraction was performed using the DNeasy Blood and Tissue (Qiagen) and Wizard® Genomic DNA Purification (Promega) kits, according to the manufacturer's instructions. Next, we amplified a 460 bp fragment of the 16S rDNA gene with primers 16S F 5′-CCGGTCTGAACTCAGATCAAGT-3′ and 16S R 5′-CTGCTCAATGATTTTTTAAATTGCTGTGG-3' (Norris et al., 1996; Mangold et al., 1998), as well as a 700 bp fragment of the cytochrome oxidase subunit I (COI) using primers LCO1490 F 5′-GGTCAACAAATCATAAAGATATTGG-3′ and HCO2198 R 5′-TAAACTTCAGGTGACCAAAAAATCA-3' (Folmer et al., 1994). The voucher specimens of the ticks were deposited in the ectoparasite collection of the Museo de Historia Natural de la Universidad de Caldas

(MHN-UCa).

For the detection and molecular identification of *Rickettsia* species, we evaluated 30 ticks (14 larvae, 16 nymphs) and amplified a ~401 bp fragment of the citrate synthase (*gltA*) gene using primers CS-78 and CS-323 (Labruna et al., 2004). The *gltA* gene is present in all species of the genus *Rickettsia*. We performed a second PCR amplification on the samples that were positive for *gltA* using primers rompB-OF and rompB-OR, proposed by Choi et al. (2005), which amplify a fragment of 511 bp of the outer membrane protein (*ompB*) that is present in *Rickettsia* species of the spotted fever group (SFG) (Choi et al., 2005). In each set of reactions, negative (ultrapure water) and positive controls for *Rickettsia vini* DNA (kindly supplied by Dr. Marcelo Bahia Labruna) were included. The PCR products were visualized through 1.0% agarose gel electrophoresis run with TBE 1X (pH 8.0) buffer at 110 V/50 mA and stained with SYBR® Safe dye. The products were visualized on a Gel Doc-It2 310 (UVP) photodocumenter and purified using Wizard® SV Gel and PCR Clean-Up System (Promega), according to the manufacturer's instructions. The purified amplicons were Sanger sequenced at Macrogen Inc. (South Korea). The quality of the sequences was analyzed using Geneious Prime® 2019.1.3 and sequence alignments were performed with MEGAX (Kumar et al., 2018). Nucleotide divergences were estimated in MEGA X, using the Kimura 3-parameters distance model (Tamura, 1992). Species confirmation was performed using a Maximum Likelihood (ML) similarity analysis using the Kimura 3-parmeteres distance model and 1000 iterations in MEGA X. The alignment included 13 different sequences gathered from GenBank, where a total of 456 unequivocally nucleotide sites of the rickettsial outer membrane protein (*ompB*) gene were aligned. We identified and analyzed the tick and *Rickettsia* species based on similarity comparisons to public sequences in GenBank and BOLD (Barcode of Life Data Systems) databases. The sequences obtained in this study were deposited in GenBank.

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3. Results

We captured 606 birds belonging to 25 families and 115 species (Table S1), including 105 resident species (576 individuals), two austral migratory species (two individuals), and eight boreal migratory species (28 individuals). The prevalence of tick infestation was 3.3% (20/606) and the infested individuals comprised 15 species (Table 2). We collected 465 ticks (445 larvae and 20 nymphs), all belonging to the genus *Amblyomma* (Acari: Ixodidae) (Table 2). The infested birds belonged mainly to the families Thraupidae (*Ramphocelus carbo, Sporophila angolensis, Sporophila intermedia,* and *Saltator coerulescens*), Turdidae (*Turdus leucomelas* and *Turdus ignobilis*), and Troglodytidae (*Troglodytes aedon* and *Campylorhynchus griseus*) (Table 2). The species *T. aedon* showed high parasite load (440 larvae), while we did not find tick infestation on migratory birds from the northern and southern hemispheres (Table S1). We molecularly confirmed the identity of *Amblyomma nodosum, Amblyomma longirostre*, and *Amblyomma mixtum.* It was not possible to confirm the molecular identity of six ticks (poor DNA quality); therefore, these individuals were morphologically identified (Table 2). We report 15 associations between wild birds and ticks, which involve 15 bird species and four tick species. In particular, we found eight new associations for the Americas (Table 2), *T. aedon, Icterus chrysater, S. angolensis, S. intermedia, S. coerulescens* infested with *A. nodosum*, *Ortalis ruficauda* infested with *A. mixtum*, and *Camptostoma obsoletum* with A. *longirostre*. Furthermore, *C. griseus* is documented, for the first time infested by *Amblyomma* ticks.

We detected a prevalence of 6.6% (2/30) of *Rickettsia* infection in the tick species *A. nodosum* (Table 3). The partial gene sequences of *gltA* and *ompB* for *Rickettsia parkeri* strain Atlantic rainforest showed both 100% identity to the corresponding sequences available in GenBank (Table 3). The phylogenetic analysis based on the *ompB* gene (Fig. 2) nested our sequences with *R. parkeri* strain Atlantic rainforest, *R. parkeri* strain Portsmouth, *R parkeri* s. s and *R. africae* (bootstrap support: 82%).

The infected *A. nodosum* individuals were found associated with hosts *S. angolensis* and *Formicivora grisea* in localities 6 (Tame) and 8 (Arauca), respectively. The GenBank accession numbers of the nucleotide sequences obtained in this study are [MT471971-MT471980] for the 16S rRNA mitochondrial gene; [MT439632] for the COI mitochondrial gene; [MT501330-MT501331] for the citrate synthase *gltA* gene; [MT501328-MT501329] for the outer membrane protein *ompB* gene.

4. Discussion

We found eight new associations between birds and ticks, including

Table 2
Wild bird species infested with ticks in the department of Arauca and results of BLAST searches for the DNA sequences of the ticks collected in this study.

Locality[a]	Host Bird		Tick species (number of specimens/ stage)	No. infested/No. tested (%)	Closest identity (gene: accession number)
	Family				
6	Cracidae	*Ortalis ruficauda*	*Amblyomma mixtum* (2/nymphs)	1/1 (100)	*A. mixtum* [COI: MF363073.1] 100%
7,8	Thamnophilidae	*Formicivora grisea*	*Amblyomma nodosum* (4/nymphs)	2/5 (40)	*A. nodosum* [16S: MH818417.1] 99.2%
7	Furnariidae	*Dendroplex picus*	*A. nodosum* (1/larva; 1/nymphs)[b]	1/4 (25)	N.D[c]
7	Tyrannidae	*Camptostoma obsoletum*	*Amblyomma longirostre* (1/nymph)[b]	1/20 (5)	N.D[c]
6	Tytiridae	*Pachyramphus polychopterus*	*A. longirostre* (1/larva)	1/7 (14.3)	*A. longirostre* [16S: MH818419.1] 100%
7	Troglodytidae	*Troglodytes aedon*	*A. nodosum* (440/larvae)[d]	2/10 (20)	*A. nodosum* [16S: FJ424402.1] 99.6%
4		*Campylorhynchus griseus*	*Amblyomma* sp. (2/larva)[b]	1/4 (25)	N.D[c]
3	Turdidae	*Turdus leucomelas*	*A. nodosum* (1/nymphs)	1/8 (12.5)	*A. nodosum* [16S: MH818417.1] 99.7%
6		*Turdus ignobilis*	*A. nodosum* (1/nymphs)	1/7 (14.3)	*A. nodosum* [16S: MH818417.1] 99.7%
6	Fringillidae	*Euphonia laniirostris*	*Amblyomma* sp. (1/larva)[b]	1/5 (20)	N.D[c]
6	Icteridae	*Icterus chrysater*	*A. nodosum* (1/nymph)	1/2 (50)	*A. nodosum* [16S: KP686064.1] 100%
7,8	Thraupidae	*Ramphocelus carbo*	*A. nodosum* (2/nymphs)	2/13 (15.4)	*A. nodosum* [16S: FJ424402.1] 100%
6		*Sporophila angolensis*	*A. nodosum* (3/nymphs)	2/5 (40)	*A. nodosum* [16S: MH818417.1] 99.5%
7		*Sporophila intermedia*	*A. nodosum* (1/nymph)	1/19 (5.3)	*A. nodosum* [16S: FJ424402.1] 99.6%
7,8		*Saltator coerulescens*	*A. nodosum* (3/nymphs)	2/9 (22.2)	*A. nodosum* [16S: FJ424402.1] 99.6%
			Total: 465 Ticks (445 larvae, 20 nymphs)		

[a] Locality numbers indicated in Table 1 and Fig. 1.
[b] Identification by external morphology.
[c] N.D: Not Done (poor DNA quality).
[d] Larvae were morphologically assigened to the same morphotype and a selected number of individuals were randomly selected for molecular identification.

Table 3
Tick species infected with *Rickettsia* in the department of Arauca and results of BLAST searches for the DNA sequences of the rickettsiae detected in this study.

Host Bird	Tick species	No. infected/ No. tested (%)	Closest identity (%) in GenBank (accession number) according to the *Rickettsia* gene	
			gltA	ompB
F. grisea	*A. nodosum*	1/30 (3.33)	*R. parkeri* strain Atlantic rainforest [MN027564] 100%	*R. parkeri* strain Atlantic rainforest [CP040325] 100%
S. angolensis	*A. nodosum*	1/30 (3.33)	*R. parkeri* strain Atlantic rainforest [MN027564] 100%	*R. parkeri* strain Atlantic rainforest [CP040325] 100%

five between bird species *T. aedon*, *I. chrysater*, *S. angolensis*, *S. intermedia*, *S. coerulescens* and the tick species *A. nodosum*, as well as two associations between *O. ruficauda* and *C. obsoletum* and ticks *A. mixtum* and *A. longirostre,* respectively. Moreover, we provide the first report of infestation of *C. griseus* with ticks of the genus *Amblyomma.* On the other hand, seven interactions found here were previously reported in the literature (Tolesano-Pascoli et al., 2010; Ogrzewalska et al., 2011b; Luz et al., 2012; Pascoal et al., 2012; Torga et al., 2013; Ramos et al., 2015; Lugarini et al., 2015; Lima et al., 2018; Martínez- Sánchez, in 2020). These findings support the assumption of the important role of wild birds in the life cycle of several Neotropical tick species by serving as hosts for immature stages (Ogrzewalska et al., 2009a, 2009b; Budachetri et al., 2017; Nava et al., 2014; Lugarini et al., 2015). Additionally, the tick species *A. nodosum* and *A. longirostre* had not been recorded in the department of Arauca; therefore, our results expand the known distribution of these species in Colombia (Osorno-Mesa, 1940; Luque, 1948; Wells et al., 1981; Benavides-Montaño et al., 2018; Rivera-Páez et al., 2018a; Acevedo-Gutiérrez et al., 2020).

We found that 93% of the infested bird species were Passeriformes, which agrees with previous research conducted in Brazil (Labruna et al., 2007; Ogrzewalska et al., 2009a; Lugarini et al., 2015; Luz et al., 2017). Furthermore, these studies state that Passeriformes are important hosts for ticks, such as *A. longirostre* and *A. nodosum,* due to the high frequency in which immature stages of both tick species parasitize Passeriformes birds. Therefore, Passeriformes are considered their primary hosts (Nava et al., 2017).

In this study the birds parasitized by *A. longirostre* (*C. obsoletum* and *Pachyramphus polychopterus*) seek food in canopy and subcanopy (Hilty and Brown, 1986; Restall et al., 2007; Del Hoyo et al., 1992–2011), therefore, it has been hypothesized that this tick completes its life cycle in tree canopies (Labruna et al., 2007; Nava et al., 2017; Suzin et al., 2020). Other bird species parasitized by *A. nodosum* (Table 2) inhabit in lower strata, such as in the understory and ground (Hilty and Brown, 1986; Restall et al., 2007; Del Hoyo et al., 1992–2011), which seems to be related to tick infestation as mentioned by Labruna et al. (2007).

Other bird species belonging to the orders Gruiformes, Ciconiformes, and Galliformes were found as hosts for immature stages of ticks of the *Amblyomma cajennense* complex (Labruna et al., 2007; Ogrzewalska et al., 2009a; Acevedo-Gutiérrez et al., 2020). Similarly, we found *O. ruficauda* (Galliforme) infested with the species *A. mixtum*, which belongs to the *Amblyomma cajennense* complex. The association between *O. ruficauda* and *A. mixtum* is epidemiologically relevant since this tick species is a vector of *R. rickettsii* (Rivera-Páez et al., 2016, 2018a; Bermúdez and Troyo, 2018), one of the most pathogenic rickettsiae on the Americas (Parola et al., 2013; Labruna et al., 2014). The distribution of *O. ruficauda* in northeastern and eastern Colombia (Hilty and Brown, 1986; Ayerbe-Quiñones, 2018), might play an important role as hosts and dispersers of ticks infected with rickettsia in these regions where the presence of *R. rickettsii* and its vector ticks has been reported (Rivera-Páez et al., 2016, 2018b).

Amblyomma nodosum represented 98.5% (458/465) of the ticks found in this study, which supports the hypothesis that this species is highly associated with Passeriformes in the Neotropics (Labruna et al., 2007; Ogrzewalska et al., 2009b; Lugarini et al., 2015; Lima et al., 2018). Particularly, two individuals of the species *T. aedon* showed high parasite loads of *A. nodosum*. In this regard, several life history traits of this bird species could favor its infestation with ticks; for example, the foraging behavior in the low strata of the vegetation and the elaboration of the nests in cavities (Kroodsma and Brewer, 2005). These sites likely provide favorable microclimatic conditions for tick establishment and survival (Pfäffle et al., 2013). Additionally, the two *T. aedon* individuals infested with ticks were found during the incubation period so the larvae could have been acquired in the nest (Johnson and Albrecht, 2020; Pacejka et al., 1998). In the region, the high percentage of birds infested with immature stages of *A. nodosum* (75%) agrees with observations from other locations in America (Labruna et al., 2007; Ogrzewalska

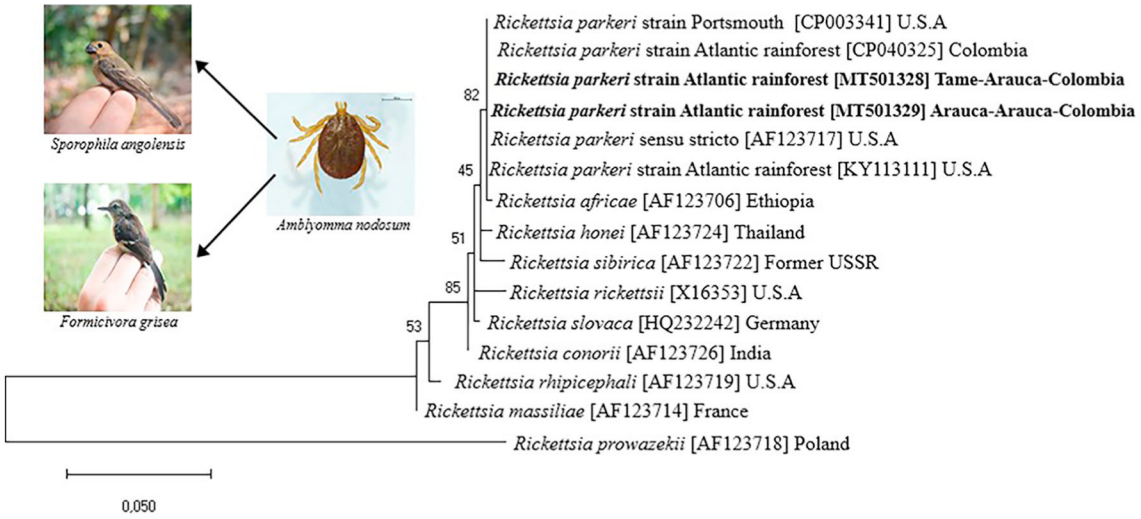

Fig. 2. Phylogenetic tree based on partial sequences of the outer membrane protein gene *ompB* present only in SFG *Rickettsia* species. The tree was inferred through Maximum Likelihood with the Tamura 3-parameter evolution model. The sequences obtained in this study appear in bold and the GenBank accessions numbers are provided within square brackets.

et al., 2009b; Lugarini et al., 2015; Lima et al., 2018). Conversely, adult ticks have been observed on mammals; for instance, in the Orinoquia, adult ticks are associated with mammal species of the order Pilosa, which are commonly found in the region (Aya-Cuero et al., 2019). These mammals are known hosts for *A. nodosum* (Luque, 1948; Witter et al., 2016; Moerbeck et al., 2018).

The detection of *R. parkeri* strain Atlantic rainforest in *A. nodosum*, which, in turn, was found infesting *F. grisea* and *S. angolensis* birds in the department de Arauca, supports the hypothesis that the Colombian Orinoquia should be considered an endemic area for rickettsial disease associated with the SFG. *Rickettsia parkeri* (strains NOD and COOPERI) were detected in *A. nodosum* collected from birds (Ogrzewalska et al., 2009b, 2011a; Lugarini et al., 2015); however, our detection of *R. parkeri* strain Atlantic rainforest in *A. nodosum* is the first association reported in the American continent for these species. *R. parkeri* strain Atlantic rainforest appears to be associated with species of the *A. ovale* complex and the NOD strain to *A. nodosum* in South America (Nieri-Bastos et al., 2018), which suggests a Neotropical origin of these strains.

Despite the distribution of *A. nodosum* includes the Neotropical region (Nava et al., 2014, 2017), there are no human reports of infestation by this ticks (Guglielmone et al., 2014; Nava et al., 2017; Moerbeck et al., 2018). In Colombia, *A. nodosum* has only been reported in the central and western areas of the country in the departments of Antioquia, Meta, Tolima, and Valle del Cauca (Osorno-Mesa, 1940; Luque, 1948; López and Parra, 1985; Benavides-Montaño et al., 2018) infesting anteaters (*Tamandua tetradactyla*). Osorno-Mesa (1940), and Martínez-Sánchez et al. (2020), reported records of infested wild birds from central Colombia. Our results extend the distribution of infested birds to the Eastern planes of the country. *R. parkeri* strain Atlantic rainforest has been recorded in Colombia in *A. ovale* ticks collected from domestic (dogs) and wild mammals (*Proechimys semispinosus*) (Londoño et al., 2014, 2017). However, no cases of rickettsial infections by this strain of *R. parkeri* have yet been reported in Colombia. In contrast, in other South American countries such as Brazil, clinical cases of *R. parkeri* strain Atlantic rainforest infections mainly associated with the transmission mediated by ticks of the *A. ovale* complex (*A. ovale* and *A. aureolatum*) have been reported in the last decade (Spolidorio et al., 2010; Nieri-Bastos et al., 2016; Da Paixão-Sevá et al., 2019). Detection of *R. parkeri* strain Atlantic rainforest in ticks that parasitize wild birds, suggests the role that these vertebrates may have for the lodging and dispersal of ticks infected with rickettsia. In this context, it is necessary to carry out studies to expand the knowledge of the associations between birds, ticks, and rickettsiae.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2020.09.001.

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3.2. CAPÍTULO II

Seroprevalence and detection of *Rickettsia* spp. in wild birds of Arauca (Colombian Orinoquia region)

Seroprevalence and detection of *Rickettsia* spp. in wild birds of Arauca (Colombian Orinoquia region)

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Abstract

Wild birds have an important role as hosts and vectors of ticks infected by rickettsiae. However, the role of birds as reservoirs of tick-borne rickettsiae is unknown and poorly understood. This is particularly relevant in the case of several tropical and subtropical areas, at a global level where migration influences the dispersal of ectoparasites and pathogens, of importance in public health. In this context, this research aimed to detect and evaluate exposure to spotted fever group rickettsiae in wild birds that could represent reservoirs in the Department of Arauca in the Colombian Orinoquia region. Sampling was conducted from November to December of 2018 and March, July and August of 2019 in three municipalities of the Department of Arauca (Colombia). Blood samples were collected from 256 birds and processed to obtained serum (n = 155) and DNA (n = 256) samples. The serum samples were processed for indirect immunofluorescence assays (IFA) against *Rickettsia rickettsii, Rickettsia parkeri, Rickettsia amblyommatis, Rickettsia rhipicephali,* and *Rickettsia bellii*. Additionally, the presence of rickettsiae was detected through PCR amplification of the citrate synthase (*gltA*) gene. The IFA results revealed seropositivity in 97 samples (62.5%), mainly in birds belonging to the families Tyrannidae, Thraupidae, and Columbidae for a total of 54 species of resident and migratory birds (boreal and austral). Only one sample, from *Phacellodomus rufifrons* was positive for *Rickettsia* with the *gltA* gene. The presence of antibodies in more than half of the individuals evaluated by IFA is an indication of a previous contact of these birds with ticks carrying rickettsiae that could infect the birds. The almost zero detection of

rickettsiae in the blood of seropositive birds is possibly due to a short period of bacteremia, which is not reached to be detected. However, experimental studies are required to improve our understanding of the role of wild birds as sources of rickettsial infections in ticks.

Keywords: Antibody, antigen, bacteremia, immune, reservoir, ticks.

1. Introduction

Wild birds are known to host and transport ticks, mainly in immature stages, which can be infected by different zoonotic pathogens, including bacteria of the genus *Rickettsia* (Bjöersdorff et al., 2001; Mukherjee et al., 2014; Scott et al., 2016; Tokarevich et al., 2018; Hoffman et al., 2020). Rickettsiae (Rickettsiales: Rickettsiaceae) are obligate intracellular agents, whose vectors are arthropods such as ticks, mites, fleas, and lice (Roux and Raoult, 2000; Hidalgo et al. 2013; Ogrzewalska and Pinter, 2016). Many tick species associated with birds that are involved in the transmission of pathogenic rickettsiae belong to the Ixodidae family (hard ticks) (Pacheco et al., 2012; Parola et al., 2013). These ticks can adhere for long periods during feeding and, therefore, transmit rickettsiae to their hosts (Pfäffle et al., 2013; Mukherjee et al., 2014; Luz et al., 2017). Furthermore, infected hosts can constitute a source of infection for other ticks (Hornok et al., 2014).

Rickettsiae are classified into four groups: i) the Typhus group (TG) with two member species, *Rickettsia prowazekii* and *Rickettsia typhi,* which are transmitted by lice and fleas, respectively; ii) the Spotted Fever group (SFG), which comprises more than 20 species, including the tick-borne *Rickettsia rickettsii* and *Rickettsia parkeri*; iii) the transitional group (TRG) that includes *Rickettsia akari* and *Rickettsia felis,* which are transmitted by mites and fleas, respectively; and iv) the ancestral group (AG),which

comprises *Rickettsia bellii* and *Rickettsia canadensis,* with unknown pathogenicity (Diop et al., 2020). Among tick-borne pathogenic rickettsiae found in birds, *R. rickettsii*, *R. parkeri* and others of unknown pathogenicity such as *R. amblyommatis, R. rhipicephali*, and *R. bellii* are a few that stand out (Sonenshine and Clifford, 1973; Ogrzewalska et al., 2012; Lugarini et al., 2015; Zeringóta et al., 2017). Several studies in Europe and America have proven the presence of rickettsiae in birds. For instance, *Rickettsia helvetica, R. felis*, and *Rickettsia* spp. have been detected in blood samples from birds using PCR (Ioannou et al., 2009; Špitalská et al., 2010; Hornok et al., 2014; Mukherjee et al., 2014; Berthová et al., 2016; Erwin et al., 2016). Similarly, serological assays have detected antibodies against SFG rickettsiae in wild and domestic birds (Vest et al., 1965; Lundgren et al., 1966; Maciel et al., 2013, 2016; Ebani et al., 2017).

In Colombia, wild mammals belonging to the orders Rodentia and Didelphimorfia have been identified as possible reservoirs of rickettsiae, in addition to several domestic animals that could represent sentinel species (Quintero et al., 2013; Riveros-Pinilla et al., 2015; Londoño et al., 2017; Arroyabe et al., 2020). In the Colombian Orinoquia region, the occurrence of ticks of medical importance such as *Amblyomma mixtum* was determined in wild animals, such as *Hydrochoerus hydrochaeris,* and domestic animals (Rivera-Páez et al., 2016; Rivera-Páez et al., 2018a). *A. mixtum* collected from *Equus caballus* and *Bos taurus* presented infection by *R. rickettsii* (Rivera-Páez et al., 2018b). Moreover, the circulation of *Rickettsia parkeri* sensu lato, was detected in *Amblyomma nodosum* collected from wild birds (*Formicivora grisea* and *Sporophila angolensis*) (Cardona-Romero et al., 2020). Detection of antibodies against rickettsiae in humans and domestic animals, in addition to a SFG-rickettsiosis case description in the Orinoquia region (Miranda et al., 2011; Riveros-Pinilla et al., 2011; Gómez-Quintero et al., 2017)

have demonstrated that this region can be considered another endemic region for SFG in Colombia (Rivera-Páez et al., 2018b). Additionally, the Colombian Orinoquia is home to mammals such as *Odocoileus cariacou, Hydrochoerus hydrochaeris, Proechimys* sp*.,* and *Didelphis marsupialis,* which can be potential reservoirs of rickettsiae (Labruna et al., 2009; Quintero-Vélez et al., 2012; Quintero et al., 2013; Sayler et al., 2016; Londoño et al., 2017).

In Colombia, several wild bird species are known as hosts of ticks infected by SFG rickettsiae (Cardona-Romero et al., 2020; Martínez-Sánchez et al., 2020); however, the role of birds as sources of rickettsial infections is unknown. Elfving et al. (2010) mention that the effectiveness of birds as reservoirs of rickettsiae is unclear. Some vertebrate hosts can present infections that are transmitted to uninfected ticks, while other hosts produce very low or transient rickettsemias that are not enough to infect ticks (Elfving et al., 2010; Hornok et al., 2014). The role of wild birds as potential reservoirs involved in the epidemiology of rickettsial diseases is not understood (Ogrzewlaska et al., 2008; Pacheco et al., 2012; Ogrzewlaska and Pinter, 2016); therefore, this is an actual topic of study and debate. This study was aimed to detect and evaluate exposure of wild birds to SFG rickettsiae and their reservoir role in the Colombian Orinoquia region.

2. Materials and methods

2. 1. Study area and sample collection

This study was conducted in the municipalities of Arauca, Tame, and Cravo Norte in the Department of Arauca located in the Colombia Orinoquia region. The study area spanned flooded savannas and gallery forests in the municipalities of Arauca (06°36'40" N 70°31'51" W, 06°46'39" N 70°42'25" W) and Cravo Norte (06°32'15" N 70°31'14" W) and an area of agropecuary use in the municipality of Tame (06°24'52" N 71°32'04" W).

The sampling was done from November to December of 2018 and in March, July, and August of 2019, at an elevational range from 120 to 234 meters above sea level (m a.s.l.), during the dry (December and March) and wet seasons (July and August).

Blood samples from wild birds were collected through brachial venipuncture in 60 µL microhematocrit tubes without anticoagulant (Maciel et al., 2013). Approximately 40 µL of each blood sample were deposited in FTA cards (Whatman Ltda., England) for DNA conservation and subsequent use in molecular assays for detection of rickettsiae. The remaining blood samples were refrigerated for five hours, then, centrifugated to separate the serum fraction and stored at -70°C. The blood and serum samples were conserved in the Genetics laboratory and the laboratory of the group in Biohealth Research of Universidad de Caldas. This study was approved by the bioethics committee of the Faculty of Exact and Natural Sciences of Universidad de Caldas and developed under the framework permit granted to Universidad de Caldas by the *Autoridad Nacional de Licencias Ambientales* (ANLA) of Colombia (Resolution 02497 of December 31st of 2018).

2.2. Indirect immunofluorescence assay

The serum samples were analyzed using indirect immunofluorescence assay (IFA) according to the methodology proposed by Horta et al. (2004). Briefly, sera were diluted in 2-fold increments with phosphate-buffered saline (PBS), starting from the 1:64 dilution, using crude antigens derived from five rickettsiae isolates: *R. rickettsii* (strain Taiaçu)*, R. parkeri* (strain AT24)*, R. amblyommatis* (strain Ac37)*, R. rhipicephali* (strain HJ5)*,* and *R. bellii* (strain Mogi), which were fixed on slides for IFA. The slides were prepared at the Laboratório de Doenças Parasitárias do Departamento de Medicina Veterinária Preventiva e Saúde Animal da Faculdade de Medicina Veterinária e

Zootecnia, Universidade de São Paulo (USP-Brazil). Antibodies were detected against fluoresceinated goat anti-chicken IgY (Jackson ImmunoResearch Laboratories) diluted at 1:400 and visualized under an ultraviolet light microscope at 400X at the Laboratório de Doenças Parasitárias do Departamento de Medicina Veterinária Preventiva e Saúde Animal da Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo. Each microscope slide included a previously determined non-reactive serum (negative control) and a reactive serum (positive control). Serum samples that were reactive to at least one out of five antigens in the initial dilution were considered positive and were subsequently diluted to 1:1024 for titration. An endpoint titer at least 4-fold higher for a *Rickettsia* species than that observed for other *Rickettsia* species was considered probably homologous to the first *Rickettsia* species or a very closely related species (Horta et al., 2004; Pacheco et al., 2007).

2.3. Molecular detection of Rickettsia spp.

DNA extraction for the molecular detection of rickettsiae species in the blood samples from birds was done using the Wizard® Genomic DNA Purification kit (Promega), according to the manufacturer's instructions. A fragment of 401 bp of the citrate synthase (*gltA*) gene, which is present in all *Rickettsia* species, was amplified using primers CS-78 5′-GCAAGTATCGGTGAGGATGTAAT-3′and CS-323 5′-GCTTCCTTAAAATTCAATAAATCAGGAT-3′ (Labruna et al., 2004). A negative control (ultrapure water) and positive control DNA from *Rickettsia vini* were included for each reaction. The PCR products were visualized through 1% agarose gel electrophoresis and purified using the Wizard® SV Gel and PCR Clean-Up System (Promega), according to the manufacturer's protocol. DNA sequencing was performed by Macrogen Inc. (South Korea) and the sequences were deposited in GenBank. DNA sequence quality was

assessed using Geneious Prime® 2019.1.3. The identification and analysis of the rickettsial species was based on sequence similarity to public sequences in GenBank and BOLD (Barcode of Life Data Systems, www.barcodinglife.com) databases.

3. Results

A total of 155 samples from 63 bird species were analyzed by IFA. Among the samples, 62.5% (n = 97) reacted positive in the initial dilution of 1:64 for at least one out of five *Rickettsia* species assessed. The birds were mostly members of the families Tyrannidae (11 species, 21 individuals), Thraupidae (7 species, 21 individuals) and Columbidae (6 species, 10 individuals). Three species of boreal migratory birds, *Tyrannus dominicensis* (4 individuals) of the Tyrannidae family, *Setophaga ruticilla* (1 individual) and *Setophaga petechia* (1 individual) of the Parulidae family, presented antibodies against rickettsiae. Two southern migratory species *Elaenia parvirostris* (1 individual) and *Empidonomus varius* (1 individual) of the Tyrannidae family were also seropositive for rickettsiae.

Of 97 positive samples, only 40 were titrated, since the volume of serum obtained in the remaining samples was too low. This limitation was caused by the small size of the bird species captured. Of the titrated sera, 22.5%, 17.5%, 12.5%, 10%, and 2.5% of the samples showed titers to *R. amblyommatis*, *R. parkeri*, *R. bellii*, *R. rhipicephali*, and *R. rickettsii* at least fourfold higher than to other rickettsiae antigen, respectively (Table 1). The remaining percentage (35%) of the samples presented titers that did not allow defining the species of *Rickettsia*.

Table 1. Results of the antibody titration (dilutions 1:64, 1:128, 1:256, 1:512, 1:1024) of the wild birds seropositive for rickettsiae of the spotted fever group and of the ancestral

group.

Bird family	Bird species	Titrations				
		R. rickettsii	***R. parkeri***	***R. bellii***	***R. rhipicephali***	***R. amblyommatis***
Columbidae	*Leptotila verreauxi*	(-)	(-)	(1/128)**	(-)	(-)
	Leptotila verreauxi	(-)	(1/256)	(1/128)	(-)	(1/1024)**
	Leptotila rufaxilla	(-)	(-)	(-)	(1/128)**	(-)
	Leptotila rufaxilla	(-)	(-)	(-)	(-)	(1/128)**
	Columbina talpacoti	(-)	(1/1024)**	(-)	(-)	(-)
Furnariidae	*Dendrocincla fuliginosa*	(-)	(-)	(-)	(-)	(1/1024)**
	Phacellodomus rufifrons +	(-)	(-)	(-)	(1/1024)	(1/1024)
Tyrannidae	*Elaenia chiriquensis*	(-)	(1/1024)	(1/1024)	(1/1024)	(1/1024)
	Myiozetetes cayanensis	(-)	(1/1024)	(-)	(1/1024)	(-)
	Myiozetetes cayanensis	(-)	(1/128)	(1/1024)**	(-)	(-)
	Pitangus sulphuratus	(-)	(1/128)	(1/128)	(1/512)**	(-)
	Pitangus sulphuratus	(1/128)	(1/1024)**	(-)	(1/64)	(-)
	Pitangus sulphuratus	(-)	(1/64)	(1/1024)	(-)	(1/1024)
	Empidonomus varius (AM)	(-)	(1/512)	(-)	(-)	(1/256)
	Tyrannus melancholicus	(-)	(-)	(1/256)	(1/1024)**	(-)
	Tyrannus savana	(-)	(-)	(1/1024)**	(-)	(-)
	Tyrannus dominicensis (BM)	(1/512)	(1/1024)	(1/1024)	(1/1024)	(1/1024)
	Tyrannus dominicensis (BM)	(-)	(-)	(-)	(-)	(1/1024)**
	Tyrannus dominicensis (BM)	(-)	(1/128)**	(-)	(-)	(-)
	Myiarchus tuberculifer	(-)	(1/1024)	(-)	(-)	(1/512)
Tiyiridae	*Pachyramphus polychopterus*	(-)	(1/1024)	(1/512)	(-)	(1/256)
Troglodytidae	*Campylorhynchus nuchalis*	(-)	(1/128)	(1/1024)	(1/1024)	(-)
	Campylorhynchus griseus	(-)	(-)	(1/1024)	(1/512)	(-)
	Thryophilus rufalbus	(1/256)	(1/128)	(1/128)	(1/256)	(1/256)
Turdidae	*Turdus leucomelas*	(-)	(1/1024)**	(-)	(-)	(1/256)
	Turdus leucomelas	(-)	(-)	(-)	(-)	(1/512)**
	Turdus leucomelas	(-)	(-)	(-)	(1/1024)**	(-)
	Turdus leucomelas	(-)	(-)	(-)	(-)	(1/256)**
	Turdus ignobilis	(-)	(1/1024)**	(-)	(-)	(-)
Fringillidae	*Euphonia laniirostris*	(-)	(-)	(1/128)**	(-)	(-)
	Euphonia laniirostris	(-)	(-)	(-)	(1/128)	(1/1024)**
Icteridae	*Icterus nigrogularis*	(-)	(1/1024)	(-)	(1/1024)	(-)
Thraupidae	*Ramphocelus carbo*	(-)	(1/256)**	(-)	(-)	(-)
	Ramphocelus carbo	(1/256)**	(-)	(-)	(1/64)	(-)
	Ramphocelus carbo	(1/1024)	(1/1024)	(1/1024)	(1/1024)	(-)
	Saltator orenocensis	(-)	(-)	(1/256)	(1/512)	(1/1024)
	Saltator coerulescens	(1/64)	(-)	(-)	(1/128)	(1/512)**
	Saltator coerulescens	(-)	(-)	(1/512)**	(1/64)	(-)

Bird family	Bird species	Titrations				
		R. rickettsii	***R. parkeri***	***R. bellii***	***R. rhipicephali***	***R. amblyommatis***
	Thraupis palmarum	(-)	(1/1024)**	(-)	(-)	(1/128)
	Thraupis palmarum	(-)	(-)	(-)	(-)	(1/128)**
	Total high titers	1 (2.5%)	7 (17.5%)	5 (12.5%)	4 (10%)	9 (22.5%)

+ Bird species positive for *Rickettsia* by PCR
**High titer
(-) Negative titer
AM: Austral migratory
BM: Boreal migratory

In total, 256 blood samples were analyzed by PCR; however, only one sample was positive for *Rickettsia* with the *gltA* gene (the amplification of the *ompA* and *ompB* genes was achieved, but good quality sequences were not obtained). This positive sample corresponded to an individual of the family Furnariidae, species *Phacellodomus rufifrons*. Observations made in the field showed that this individual had necrosis and total loss of movement of the lower right limb. The *Rickettsia*-positive specimen was deposited in the Bird Collection of the Museum of Natural History of Universidad de Caldas under collection code MCR001 (Figure 1). The GenBank accession number of the citrate synthase (*gltA*) gene nucleotide sequence generated in this study is [MW314090].

4. Discussion

In the present study, 62.5% of wild birds, assessed by IFA, presented antibodies against rickettsiae. Lundgren et al. (1966), using the complement fixation technique (CF), found antibodies against rickettsiae in a lower percentage (5.5%) of the evaluated birds. Domestic pigeons (*Columba livia*) and falcons (*Circus cyaneus*, *Buteo jamaicensis*) that had been experimentally infected with *R. rickettsii* presented antibodies against

rickettsiae. Other studies by Kelly et al. (1996), Maciel et al. (2013, 2016) and Ebani et al. (2017) detected seropositivity against SFG in domestic and wild bird species by IFA. Species of the families Tyrannidae, Thraupidae, and Columbidae showed the greatest number of individuals with antibodies against rickettsiae. These bird families have been reported to be infested by ticks infected with SFG (e.g., *R. amblyommatis, R. parkeri*) and the ancestral group (*R. bellii*) (Ogrzewalska et al., 2009; Ogrzewalska et al., 2012; Luz et al., 2017).

The migratory boreal species *T. dominicensis* of the family Tyrannidae and the species *S. ruticilla* and *S. petechia* of the family Parulidae presented antibodies against rickettsiae. Species of these families of birds had already presented infestation by ticks infected by rickettsiae from the SFG in Colombia (Martínez-Sánchez et al., 2020). Austral migratory species such as *E. parvirostris* and *E. varius* of the Tyrannidae family were seropositive for rickettsiae. Although these two migratory species have not been reported to be infested by ticks infected with rickettsiae, the detection of the presence of antibodies is an indication of a previous contact of these birds with ticks carrying rickettsiae that could infect the bird (Maciel et al., 2013).

The highest percentage of the titers was for *R. amblyommatis*, a *Rickettsia* sp. that infects different species of ticks of the genus *Amblyomma*, including some associated with birds (e.g., *Amblyomma longirostre*, *Amblyomma varium*, *Amblyomma calcaratum*, *Amblyomma geayi*, *A. nodosum*) (Budachetri et al., 2017; Luz et al., 2017; Bermúdez et al., 2020; Martínez-Sánchez et al., 2020). *R. amblyommatis* probably represents the most prevalent and widely distributed SFG species in the Americas (Karpathy et al., 2016). Therefore, it is possible that the sampled birds have been parasitized by ticks infected with this rickettsia at some point. Wild birds also showed high titers against *R. parkeri*,

where *R. parkeri* sensu lato had already been reported *A. nodosum* infesting of wild birds in the study area (Cardona-Romero et al., 2020). Therefore, it is possible that *R. parkeri* is circulating among ticks and birds in the Orinoquia region. The high titers for other rickettsiae species such as *R. rhipicephali*, *R. rickettsii* and *R. bellii* are possibly due to the circulation of these rickettsiae in the Orinoquia region. However, only the circulation of *R. rickettsii* and another SFG species (*Candidatus* Rickettsia colombianensi) has been demonstrated in this region (Riveros-Pinilla et al., 2015; Gómez-Quintero et al 2017; Rivera-Páez et al., 2018b; Sánchez -Lerma et al., 2019). Different birds presented the same titers for two or more of the evaluated rickettsiae, possibly due to the cross-reactivity of antibodies that can be generated when evaluating by IFA, making difficult the serological identification of the *Rickettsia* spp. involved in a possible infection (Horta et al., 2004).

The PCR assays detected a single individual positive for rickettsiae (0.4%). Similar results were found in other studies that aimed to detect rickettsiae in birds (Stańczak et al., 2009; Cohen et al., 2015; Budachetri et al., 2017). Likewise, Ioannou et al. (2009), Hornok et al. (2014), Berthová et al. (2016), Erwin et al. (2016) found a prevalence lower than 9% for rickettsiae in the blood of wild birds. This low prevalence of rickettsiae in the blood may suggest a low efficienty in the transmission of these pathogens to ticks (Lundgren et al., 1966; Hornok et al., 2014; Ogrzewalska and Pinter, 2016). However, this must be confirmed by future studies.

The *gltA* gene sequence amplified from the blood of *P. rufifrons*, this finding report of the molecular detection of a *Rickettsia* sp. in this bird species. *Rickettsia helvetica* was detected in Europe in blood samples from robin (*Erithacus rubecula*), dunnock (*Prunella modularis*) (Hornok et al., 2014), and other bird species in Europe (Berthová et al., 2016).

Rickettsia felis was detected in North America in a crested caracara (*Caracara cheriway*) (Erwin et al., 2016). However, future studies are necessary for the confirmation and complete molecular characterization of samples from wild birds.

The presence of immunoglobulins Y (IgY) at a dilution of 1:1024 for *R. rhipicephali* and *R. amblyommatis* and the presence of *Rickettsia* sp. in the blood of *P. rufifrons* possibly indicate a recent infection since the individual showed high titers. Future studies should aim to conduct experiments in wild birds in order to improve the understanding of the role of birds in the transmission of rickettsiae.

This study demonstrates seropositivity against SFG rickettsiae in more than half of the individuals evaluated by IFA, as an indication of a previous contact of these birds with ticks carrying rickettsiae that could infect the birds. The almost zero detection of rickettsiae in the blood of seropositive birds is possibly due to a short period of bacteremia. However, this fact remains unclear few information is available on the role of wild birds as competent reservoirs of rickettsiae and their role in the transmission and epidemiology of these pathogenic bacteria.

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1. CONSIDERACIONES FINALES

Diferentes autores han dado a conocer con sus estudios el importante rol de las aves como hospederas de garrapatas vectoras de enfermedades. Donde gracias a su capacidad de vuelo y a procesos de migración de largas distancias, las aves silvestres se han visto involucradas en la dispersión y distribución de garrapatas infectadas por diferentes patógenos. En Colombia el país con la mayor diversidad de aves en el mundo, este es el segundo estudio que identifica las asociaciones entre garrapatas infectadas por bacterias del género *Rickettsia* y aves silvestres, y es el primer estudio que ha buscado detectar la presencia de rickettsias en aves silvestres como posibles reservorios. Gracias a esta investigación desarrollada en la Orinoquía colombiana, se logró establecer la presencia en esta región de la segunda *Rickettsia* más patógena en el continente americano *Rickettsia parkeri* strain Atlantic rainforest en garrapatas asociadas a aves silvestres. De igual manera, la detección de anticuerpos frente a rickettsias del grupo de las fiebres manchadas en aves silvestres en los municipios de Arauca, Tame y Cravo Norte, y la detección en sangre *Rickettsia* sp. en Tame, son indicios de la circulación de agentes rickettsiales en la región.

Estos resultados refuerzan la idea de considerar la Orinoquía como la tercera región endémica para rickettsias del grupo de las fiebres manchadas (SFG) en Colombia. En consecuencia, la presencia de *R. parkeri* strain Atlantic rainforest en los municipios de Arauca y Tame debe de ser tenida en cuenta por las entidades territoriales de salud, para la focalización de posibles brotes de rickettsiosis.

Este estudio permitió ampliar el conocimiento acerca de las asociaciones entre especies de garrapatas neotropicales y aves silvestres en Colombia y en el continente Américano. De igual manera, fue posible establecer una nueva asociación entre la garrapata neotropical *A. nodosum* con la cepa de rickettsia patógena *R. parkeri* strain Atlantic rainforest en América.

Los resultados de esta investigación indican que es necesario involucrar otros taxones

silvestres (e.g., mamíferos), que participen en el ciclo de vida de las garrapatas encontradas en la zona de estudio. Dado que estos taxones podrían estar actuando como reservorios o amplificadores de rickettsias patógenas en la región.

Diversas especies de aves presentaron anticuerpos frente a rickettsias y solo una especie de ave presentó infección o bacteriemia por una *Rickettsia.* Las especies seropositivas tanto residentes como migratorias (boreales y australes), al parecer, en algún momento adquirieron rickettsias, lo que les permitió generar una respuesta inmune frente a estas. La prevalencia casi nula de rickettsias en sangre de aves seropositivas, posiblemente se deba a niveles bajos de parasitemia o a infecciones transitorias que no alcanzan a ser detectadas en sangre. Dado que, inicialmente las rickettsias invaden las células endoteliales y solo en una etapa más avanzada de la infección con alta parasitemia, logran pasar al torrente sanguíneo.

La detección de una especie de *Rickettsia* y la detección de anticuerpos del SFG y del GA en el presente estudio, demuestran la circulación de estos agentes rickettsiales en la zona muestreada. Aunque, debido a las características antigénicas compartidas entre especies de rickettsias, es posible que se presenten reacciones cruzadas entre el mismo grupo de rickettsias (SFG), lo que puede dificultar su identificación.

Se puede esperar que las especies de aves evaluadas en este estudio tengan una baja eficacia en la trasmisión de rickettsias a las garrapatas que hospedan. Aunque, posiblemente esto cambie de acuerdo con las condiciones como especie, edad, sexo y estado inmunológico de los individuos. Para dar mayor claridad en este aspecto, es necesario el desarollo de estudios experimentales futuros con diferentes especies de aves, que permitan mejorar la comprensión del papel de las aves silvestres como reservorios de rickettsias o posiblemente de su actuación como refractarias ante la presencia de estas bacterias.

Finalmente, esta investigación provee información base sobre la infestación por garrapatas infectadas por rickettsias en aves silvestres y del papel de las aves como

posibles fuentes o reservorios de dichos microorganismos en Colombia. Esto es un punto de partida para futuras investigaciones que deben ser priorizadas en estos hospederos silvestres, dado el alto potencial de las aves en la distribución y transporte de garrapatas infectadas por patógenos. Además de los procesos de migración por aves, en los que Colombia es clave al ser sitio de paso o de invernada para diferentes especies provenientes del hemisferio Norte y Sur de América.

5. APÉNDICES

5.1. Appendix A. Supplementary data Chapter 1

Table S1. Wild bird species examined in the localities sampled and prevalence of infestation with ticks.

Municipality	Locality[a]	Host Bird			No. infested/No. tested (%)
		Family	Species	Residence status	
Tame	6	Cracidae	*Ortalis ruficauda*	R	1/1 (100)
Arauca, Cravo Norte	3,4,5,7,8	Columbidae	*Leptotila verreauxi*	R	0/9 (0)
Tame	6		*Leptotila rufaxilla*	R	0/2 (0)
Arauca	2		*Zenaida auriculata*	R	0/2 (0)
Arauca	2,4,8		*Columbina minuta*	R	0/5 (0)
Arauca, Tame	3,6,7		*Columbina talpacoti*	R	0/4 (0)
Arauca, Cravo Norte	2,4,5,8		*Columbina squammata*	R	0/9 (0)
Arauca	8		*Claravis pretiosa*	R	0/1 (0)
Arauca	8	Cuculidae	*Crotophaga ani*	R	0/1 (0)
Arauca	8		*Crotophaga sulcirostris*	R	0/1 (0)
Arauca, Tame, Cravo Norte	3,5,6	Caprimulgidae	*Nyctidromus albicollis*	R	0/3 (0)
Arauca, Tame	6,7	Trochilidae	*Glaucis hirsutus*	R	0/4 (0)
Arauca, Tame	6,8		*Phaethornis anthophilus*	R	0/10 (0)
Arauca, Tame	1,6,7		*Chlorostilbon mellisugus*	R	0/4 (0)
Arauca, Tame	2,6,8		*Chrysuronia versicolor*	R	0/10 (0)
Arauca	2,6,8		*Chionomesa fimbriata*	R	0/19 (0)
Tame	6		*Saucerottia viridigaster*	R	0/2 (0)
Arauca	8	Charadriidae	*Vanellus chilensis*	R	0/1 (0)
Arauca	8	Burhinidae	*Burhinus bistriatus*	R	0/1 (0)
Arauca	2	Threskiornithidae	*Mesembrinibis cayenensis*	R	0/1 (0)
Tame	6	Strigidae	*Megascops choliba*	R	0/1 (0)
Arauca, Tame	3,6,7,8	Alcedinidae	*Chloroceryle aenea*	R	0/6 (0)
Arauca	7		*Chloroceryle americana*	R	0/1 (0)
Tame	6		*Chloroceryle inda*	R	0/1(0)
Arauca, Tame	6,7	Galbulidae	*Galbula ruficauda*	R	0/8 (0)
Arauca	8		*Hipnelus ruficollis*	R	0/1 (0)
Tame	6	Picidae	*Picumnus squamulatus*	R	0/3 (0)
Arauca	2		*Melanerpes rubricapillus*	R	0/1 (0)
Tame	6		*Veniliornis passerinus*	R	0/3 (0)

Municipality	Locality[a]	Host Bird			No. infested/No. tested (%)
		Family	Species	Residence status	
Arauca	1,2,7		*Colaptes punctigula*	R	0/4 (0)
Arauca	7	Psittacidae	*Brotogeris jugularis*	R	0/1 (0)
Tame	6		*Forpus conspicillatus*	R	0/3 (0)
Arauca	8		*Eupsittula pertinax*	R	0/2 (0)
Tame	6	Thamnophilidae	*Sakesphorus canadensis*	R	0/1 (0)
Tame	6		*Thamnophilus doliatus*	R	0/1 (0)
Arauca	7,8		*Formicivora grisea*	R	2/5 (40)
Tame	6	Furnariidae	*Dendrocincla fuliginosa*	R	0/7 (0)
Arauca, Tame	6,7		*Dendroplex picus*	R	1/4 (25)
Tame	6		*Lepidocolaptes souleyetii*	R	0/3 (0)
Tame	6		*Phacellodomus rufifrons*	R	0/2 (0)
Arauca	1		*Cranioleuca vulpina*	R	0/1 (0)
Arauca	1		*Certhiaxis cinnamomeus*	R	0/2 (0)
Tame	6	Tyrannidae	*Tyrannulus elatus*	R	0/1 (0)
Arauca	7		*Elaenia flavogaster*	R	0/5 (0)
Arauca	8		*Elaenia parvirostris*	Ma	0/1 (0)
Arauca	7,8		*Elaenia chiriquensis*	R	0/5 (0)
Arauca, Tame, Cravo Norte	1,2,3,5,6,7,8		*Camptostoma obsoletum*	R	1/20 (5)
Arauca	6,8		*Capsiempis flaveola*	R	0/6 (0)
Arauca	8		*Inezia caudata*	R	0/4 (0)
Tame	6		*Atalotriccus pilaris*	R	0/5 (0)
Tame	6		*Poecilotriccus sylvia*	R	0/6 (0)
Arauca, Tame	1,2,3,6,8		*Todirostrum cinereum*	R	0/20 (0)
Arauca, Tame, Cravo Norte	1,3,5,6,8		*Tolmomyias flaviventris*	R	0/14 (0)
Arauca	1,3		*Cnemotriccus fuscatus*	R	0/1 (0)
Arauca	1		*Pyrocephalus rubinus*	R	0/1 (0)
Arauca	2,3		*Machetornis rixosa*	R	0/2 (0)
Arauca, Cravo Norte	2,3,5,7,8		*Myiozetetes cayanensis*	R	0/10 (0)
Arauca	2,4		*Phelpsia inornata*	R	0/2 (0)
Arauca, Cravo Norte	2,4,5,7,8		*Pitangus sulphuratus*	R	0/14 (0)
Arauca	3		*Pitangus lictor*	R	0/1 (0)
Arauca, Cravo Norte	5,6		*Myiodynastes maculatus*	R	0/2 (0)
Arauca	7		*Empidonomus varius*	Ma	0/1 (0)
Arauca	2,7		*Tyrannus melancholicus*	R	0/8 (0)
Arauca	2		*Tyrannus savana*	R	0/8 (0)
Arauca	2		*Tyrannus dominicensis*	Mb	0/16 (0)
Arauca, Tame, Cravo Norte	4,5,6		*Myiarchus tuberculifer*	R	0/6 (0)
Arauca	4		*Myiarchus venezuelensis*	R	0/2 (0)

Municipality	Locality[a]	Host Bird			No. infested/No. tested (%)
		Family	Species	Residence status	
Arauca, Cravo Norte	4,5		*Myiarchus tyrannulus*	R	0/2 (0)
Arauca	7	Tityridae	*Pachyramphus rufus*	R	0/1 (0)
Arauca, Tame	6,7		*Pachyramphus polychopterus*	R	1/7 (14.3)
Arauca	2,3	Vireonidae	*Cyclarhis gujanensis*	R	0/3 (0)
Arauca,Tame	6,8		*Pachysylvia aurantiifrons*	R	0/2 (0)
Arauca	3		*Vireo flavoviridis*	Mb	0/1 (0)
Tame	6		*Vireo altiloquus*	Mb	0/2 (0)
Arauca, Tame, Cravo Norte	5,6,7	Troglodytidae	*Troglodytes aedon*	R	2/10 (20)
Arauca,Tame	6,8		*Campylorhynchus nuchalis*	R	0/4 (0)
Arauca, Cravo Norte	2,4,5,8		*Campylorhynchus griseus*	R	1/4 (25)
Arauca	6		*Thryophilus rufalbus*	R	0/2 (0)
Arauca	4	Turdidae	*Catharus ustulatus*	Mb	0/1 (0)
Arauca, Tame	3,6		*Turdus leucomelas*	R	1/8 (12.5)
Arauca,Tame, Cravo Norte	3,4,5,6,7,8		*Turdus nudigenis*	R	0/19 (0)
Arauca, Tame	6		*Turdus ignobilis*	R	1/7 (14.3)
Arauca	2,3	Mimidae	*Mimus gilvus*	R	0/6 (0)
Arauca, Tame, Cravo Norte	5,6,8	Fringillidae	*Euphonia chlorotica*	R	0/3 (0)
Tame	6		*Euphonia laniirostris*	R	1/5 (20)
Arauca	2,4	Passerellidae	*Ammodramus humeralis*	R	0/4 (0)
Tame	6		*Arremonops conirostris*	R	0/1 (0)
Tame	6		*Arremon taciturnus*	R	0/5 (0)
Tame	6	Icteridae	*Cacicus solitarius*	R	0/1 (0)
Tame	6		*Icterus chrysater*	R	1/2 (50)
Arauca	2,4,7,8		*Icterus nigrogularis*	R	0/7 (0)
Arauca	1,7		*Gymnomystax mexicanus*	R	0/2 (0)
Arauca	1		*Quiscalus lugubris*	R	0/2 (0)
Arauca	3	Parulidae	*Parkesia noveboracensis*	Mb	0/1 (0)
Tame	6		*Setophaga ruticilla*	Mb	0/5 (0)
Tame	6		*Setophaga petechia*	Mb	0/1 (0)
Tame	6		*Setophaga striata*	Mb	0/1 (0)
Arauca	7	Thraupidae	*Nemosia pileata*	R	0/1 (0)
Arauca	2		*Sicalis citrina*	R	0/8 (0)
Arauca	1,2		*Sicalis columbiana*	R	0/33 (0)
Arauca, Tame	2,6,7		*Sicalis flaveola*	R	0/8 (0)
Tame	6		*Volatinia jacarina*	R	0/2 (0)
Arauca, Tame	6,7,8		*Ramphocelus carbo*	R	2/13 (15.4)
Arauca, Tame	1,2,6		*Sporophila minuta*	R	0/10 (0)
Arauca	2		*Sporophila angolensis*	R	2/5 (40)

Municipality	Locality[a]	Host Bird		No. infested/No. tested (%)
	Family	Species	Residence status	
Arauca, Tame	1,3,6,7,8	*Sporophila intermedia*	R	1/19 (5.3)
Arauca	1	*Sporophila plumbea*	R	0/1 (0)
Arauca	7	*Saltator orenocensis*	R	0/3 (0)
Arauca, Cravo Norte	3,4,5,7,8	*Saltator coerulescens*	R	2/9 (22.2)
Arauca, Tame, Cravo Norte	3,5,6,7,8	*Coereba flaveola*	R	0/27 (0)
Arauca,Tame, Cravo Norte	2,3,5,6,8	*Paroaria nigrogenis*	R	0/10 (0)
Arauca	1,3	*Stilpnia cayana*	R	0/3 (0)
Tame	6	*Stilpnia cyanicollis*	R	0/5 (0)
Arauca, Tame	2,4,6,7	*Thraupis episcopus*	R	0/14 (0)
Tame	6	*Thraupis palmarum*	R	0/4 (0)
			Total prevalence of ticks in birds	20/606 (3.3)

R: Residents
Ma: Austral migrants
Mb: Boreal migrants
[a]Locality numbers indicated in Table 1 and Fig. 1

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