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Migratory and Carnivorous Birds in Brazil: Reservoirs for *Anaplasma* and *Ehrlichia* Species?

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Abstract

In order to investigate new hosts for Anaplasmataceae agents in Brazil, we collected blood samples from 21 wild birds. Using molecular techniques, we detected the presence of *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*, and an *Ehrlichia* species closely related to *Ehrlichia canis* in carnivorous avian blood samples. In addition, an *Ehrlichia* species closely related to an *Ehrlichia* species found in wild felines in Brazil was also detected in a goose blood sample. Wild birds may play a role as carriers of Anaplasmataceae agents in Brazil.

Key Words: *Anaplasma phagocytophilum*—Brazil—*Ehrlichia chaffeensis*—*Ehrlichia* spp.—Wild birds.

Anaplasmataceae agents comprise obligate intracellular bacteria that can cause disease in humans and animals, whose cycle in the environment involves complex interactions between invertebrate vectors and vertebrate hosts (Dumler et al. 2001). In Brazil, *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* have been detected in Brazilian marsh deer (*Blastocerus dichotomus*) (Machado et al. 2006) and dogs (Santos et al. 2011), respectively. Regarding the occurrence of ehrlichiosis among humans in Brazil, only serological studies have been reported (Calic et al. 2004; Costa et al. 2005). Among wildlife in Brazil, *Ehrlichia* spp. have also been detected in wild felines (André et al. 2010; Widmer et al. 2011). Recently, *Rickettsia amblyommii* was detected in *Amblyomma longirostre* ticks parasitizing wild birds in a region of the Atlantic Forest, state of Bahia, Brazil (Ogrzewalska et al. 2011).

The role of birds in the epidemiology of tick-borne pathogens has been poorly studied. In the U.S., *Ixodes scapularis* tick-infested birds may play a role as carriers, and possibly reservoirs, for *Borrelia burgdorferi* (the causative agent of Lyme disease), and *Anaplasma phagocytophilum* (the causative agent of human granulocytic anaplasmosis) (Daniels et al. 2002).

Our work aimed to investigate if migratory and carnivorous birds might act as reservoirs for Anaplasmataceae agents in Brazil. Between 2009 and 2010, blood samples were collected from 21 wild birds: 8 Orinoco geese (*Neochen jubata*) (Anseriformes, Anatidae) from São Miguel do Araguaia, Mato Grosso state; 2 caracaras (*Caracara plancus*) (Falconiformes, Falconidae), 1 hawk (*Falcos sparverius*) (Falconiformes, Falconidae), 2 owls (2 *Athene cunicularia*) (Strigiformes, Strigidae), 1 barn owl (*Tytus alba*) (Strigiformes, Tytonidae), 5 vultures (*Coragyps atratus*) (Cathartiformes, Cathartidae), and 1 potoo (*Nyctibius griseus)* (Capamulgiformes, Nyctibiidae) from São Paulo state. Ectoparasites were not found parasitizing birds at the time of sample collection. DNA was extracted from blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA), and submitted to polymerase chain reaction (PCR) for Anaplasmataceae DNA, targeting 16SrRNA (Murphy et al. 1998; Massung et al. 1998; Chae et al. 2003; Headley et al. 2006), and omp-1 (Inayoshi et al. 2004) genes. For every 10 samples, a blank tube containing ultra-pure water was included in the DNA extraction to check for contamination in this process. *Ehrlichia chaffeensis* (Arkansas strain), *Anaplasma phagocytophilum* (Webster strain), and *Neorickettsia risticii* (Illinois strain) DNA-positive controls included in all PCR assays were kindly supplied by John Stephen Dumler (from Johns Hopkins School of Medicine, Baltimore, MD). In each set of reactions, five tubes containing ultra-pure water were used as controls. In order to prevent PCR contamination, DNA extraction, reaction set-up, PCR amplification, and electrophoresis were performed in separate rooms. Purified amplicons were submitted to sequencing and phylogenetic analyses using CAP3, Blast, Clustal W, and Mega4 software.

Anaplasma phagocytophilum DNA (99% identity with isolates of *A. phagocytophilum* from Russia [HQ629917], Korea [GU064899], and the Czech Republic [EU839857], based on 16S rRNA sequences) was detected in one caracara (GenBank accession no. JN217096), and two vultures (GenBank accession nos. JN217094 and JN217095) blood samples (Fig. 1).

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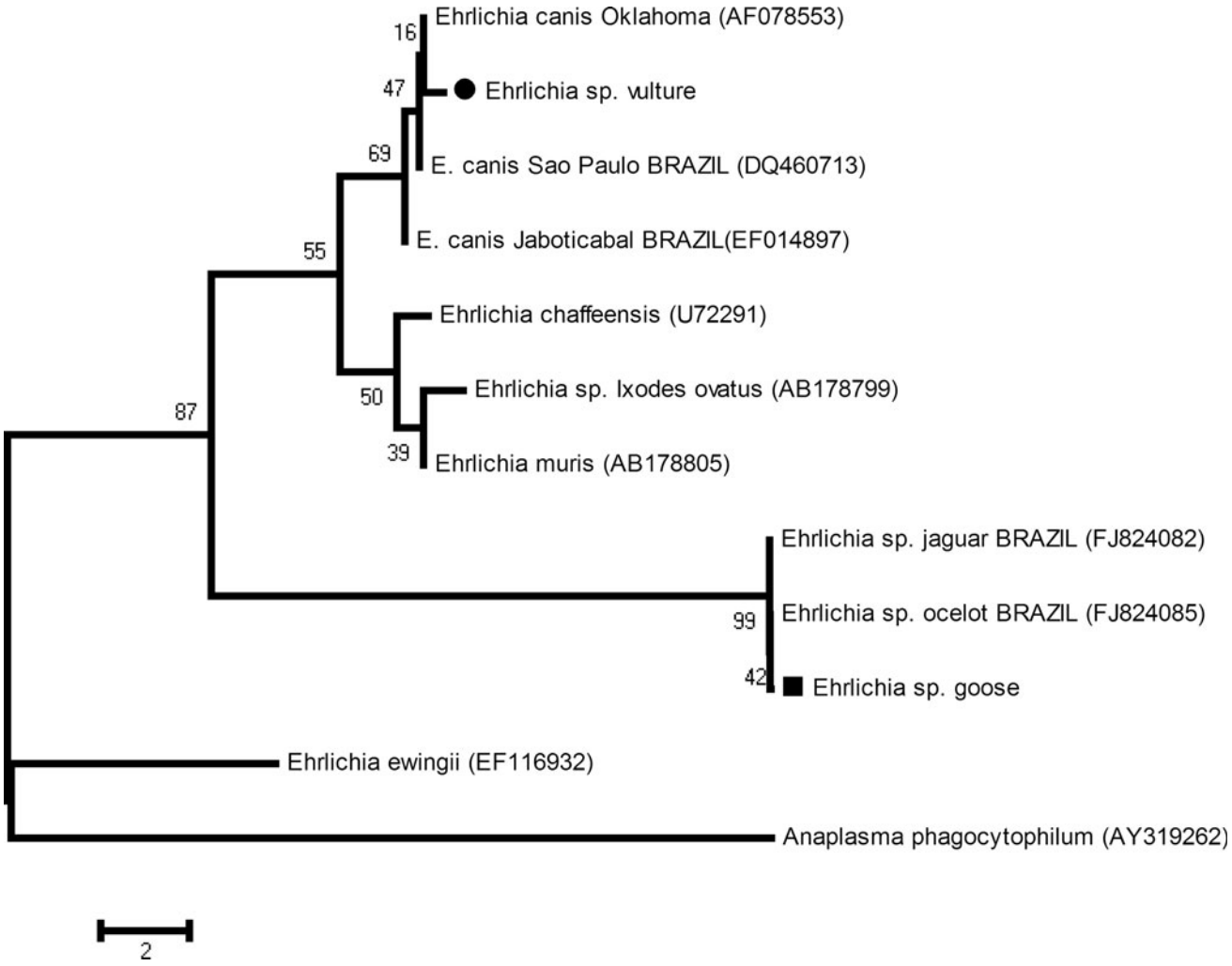

FIG. 1. Phylogenetic position of Anaplasmataceae agents isolated from Brazilian wild carnivorous and migratory birds based on 16S RNA sequences (350 bp). The tree was constructed using the neighbor-joining method, and the numbers on the tree indicate bootstrap values for the branch points. Accession numbers are indicated.

Ehrlichia chaffeensis DNA (GenBank accession no. JN217093) (100% identity with *E. chaffeensis* Brazilian marsh-deer isolate [DQ345720], China isolate [AF147752], and Arkansas isolate [CP000236], based on 16S rRNA sequences) was detected in 1 hawk. *Ehrlichia* spp. DNA was also detected in 1 vulture (GenBank accession no. JN217100) (99% similarity with *E. canis* from dogs in Brazil [EF014897] based on omp-1 sequences), and in 1 goose (GenBank accession no. JN217097) (99% similarity with *Ehrlichia* spp. found in wild felines from Brazil [FJ824085] based on omp-1 sequences) (Fig. 2).

The present work showed that birds may act as hosts for Anaplasmataceae agents in Brazil. Until now, *E. chaffeensis* and *A. phagocytophilum* have only been detected in marsh deer (*Blastocerus dichomotus*) (Machado et al. 2006), and dogs (Santos et al. 2011) in Brazil, respectively. *E. chaffeensis* DNA found in the hawk in our study was closely related to *E. chaffeensis* found in Brazilian marsh deer (Machado et al. 2006) in the 16S rRNA phylogenetic tree. The vectors involved in these cycles in the environment remains unknown.

The role of birds as reservoirs and/or carriers of tick-borne pathogens has been investigated in North America (Daniels et al. 2002; Ogden et al. 2008), and Europe (Björsdorff et al. 2001; Spitalská et al. 2006; Ioannou et al. 2009; Hildebrandt et al. 2010). Some of these works have incriminated birds only as *A. phagocytophilum*-infected tick carriers, since the agent has been detected only in nymphs (but not larvae) parasitizing sampled birds in Sweden (Björsdorff et al. 2001), Russia (Alekseev et al. 2001), Canada (Ogden et al. 2008), and Germany (Hildebrandt et al. 2010). The failure to detect *A. phagocytophilum* in larvae suggests that birds are not an important source of infection for feeding ticks, acting as incompetent reservoirs (Björsdorff et al. 2001). However, in our work, we detected the presence of *A. phagocytophilum* DNA in avian blood samples, indicating that birds may be reservoirs for this agent in Brazil. Previously, *A. phagocytophilum* was detected in *Ixodes scapularis* larvae in a veery (*Catharus fuscens*), and in American robins (*Turdus migratorius*) in the state of New York, U.S. (Daniels et al. 2002), but the authors did not rule out the possibility that the acquisition of the pathogen was via co-feeding with previously infected nymphs. On the other hand, in Poland, the detection of the same agent in quill mites from the family Syringophilidae, which parasitize avian feathers and feeds on host subcutaneous fluids, implied that they must have acquired the bacteria directly from sampled bacteremic

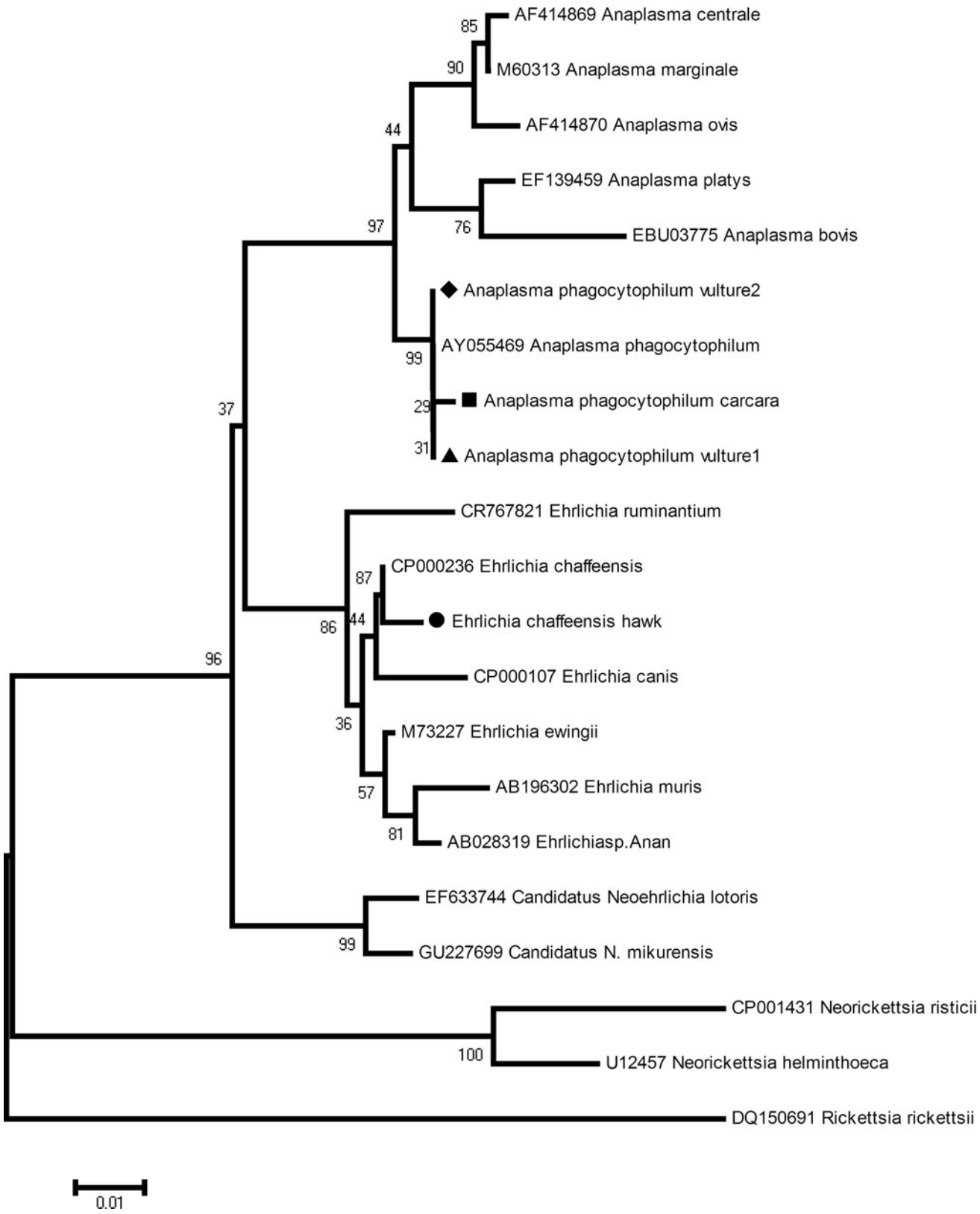

FIG. 2. Phylogenetic position of Anaplasmataceae agents isolated from Brazilian wild carnivorous and migratory birds based on omp-1 DNA sequences (300 bp). The tree was constructed using the neighbor-joining method, and the numbers on the tree indicate bootstrap values for the branch points. Accession numbers are indicated.

birds (Skoracki et al. 2006). Also, *Anaplasma* spp. DNA has been detected in wild bird blood samples in Cyprus, Greece, reinforcing the role of wild birds as reservoirs for these agents.

Also, the present work showed for the first time the detection of *E. chaffeensis* in a carnivorous bird. While knowledge about the epidemiology of this zoonotic agent in Brazil lies in its molecular detection in Brazilian marsh deer (Machado et al. 2006), and serological detection among dogs and humans (Calic et al. 2004; Costa et al. 2005), our study highlights the participation of birds in the dispersal of this pathogen, and the possibility of the establishment of new foci of infection among animals and humans. In Slovakia, an *Ehrlichia*-like species "Schotti variant" was detected in a nymph from the song thrush (*Turdus philomelos*) (Spitalská et al. 2006).

Interestingly, *Ehrlichia* species DNA closely related to an *Ehrlichia* species recently found in wild felines (André et al. 2010) was detected in an Orinoco goose, suggesting that birds may also disperse *Ehrlichia* species that circulate among wild animals in Brazil. Also, an *Ehrlichia* species closed related to *E. canis*, an endemic and widespread dog pathogen in several areas of Brazil, was found in a vulture (which was also co-infected with *A. phagocytophilum*).

Transovarial transmission of *Ehrlichia* and *Anaplasma* species has not been demonstrated, so reservoir hosts are necessary to maintain the life cycle in the environment (Dumler et al. 2001). More studies should be conducted to verify the role that birds may play as competent reservoirs for Anaplasmataceae agents through isolation of the involved agents, epidemiological studies using larger sample sizes, and experimental infections, to assess the risk that these avian species pose to human health.

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Author Disclosure Statement

No competing financial interests exist.

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