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Short communication

Molecular detection of bacteria in the families *Rickettsiaceae* and *Anaplasmataceae* in northern crested caracaras (*Caracara cheriway*)

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ABSTRACT

Bacterial pathogens of the families *Anaplasmataceae* and *Rickettsiaceae* are often spread to humans or other animals from bites from infected arthropod hosts. Recently, an increasing number of studies have implicated migratory birds in the circulation of these pathogens through the spread of arthropod vectors. However, few studies have examined the potential for resident bird populations to serve as reservoirs for these zoonoses. In this study, we used nested PCRs of the GroESL and 17 kDa genes to screen for *Anaplasmataceae* and *Rickettsiaceae*, respectively, in a resident population of the northern crested caracara (*Caracara cheriway*) from Florida ($n = 55$). Additionally, a small number ($n = 6$) of captive individuals from Texas were included. We identified one individual (1.64%) positive for *Rickettsia felis* and one (1.64%) positive for *Ehrlichia chaffeensis;* both these individuals were from Florida. Presence of these pathogens demonstrates that these birds are potential hosts; however, the low prevalence of infections suggests that these populations likely do not function as an ecological reservoir.

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Introduction

Obligate intracellular bacteria from the families *Anaplasmataceae* and *Rickettsiaceae* are the etiological agents for a variety of human and animal diseases. These bacteria use arthropods, especially, ticks, as their primary vectors for transmission (Dumler et al., 2001; Ismail et al., 2010; Parola et al., 2005). There is a growing interest, in part due to their adverse effects upon human health, in the life cycle of these pathogens in wildlife populations; especially the potential role migratory birds serve in the epidemiology of zoonoses.

Migratory birds may function as both carriers, moving infected ticks from one area to another, and as potential reservoirs of these bacteria. Migratory birds are known to be hosts of ticks infected with *Ehrlichia chaffeensis* (Alekseev et al., 2001), *Anaplasma phagocytophilum* (Alekseev et al., 2001; Björsdorff et al., 2001; Hildebrandt et al., 2010; Ogden et al., 2008), or *Rickettsia* spp (Hildebrandt et al., 2010), but usually birds act as carriers of infected ticks, as opposed to reservoirs for the bacteria.

Birds may also serve as zoonotic reservoirs. Pools of *A. phagocytophilum*-positive tick larvae (*Ixodes scapularis*) were collected from an American robin (*Turdus migratorius*) and veery (*Catharus fuscescens*) collected in New York (Daniels et al., 2002). The sampled birds were not directly tested for pathogens, but infected tick larvae, at least when transovarial transmission does not occur, indicated the pathogen was acquired from ticks feeding upon their avian hosts. However, the horizontal transfer through co-feeding of larvae and nymphs could not be completely excluded. Existing information suggests the prevalence of *Rickettsiaceae* in birds is low (3–5.5%) (Hornok et al., 2014; Ioannou et al., 2009), whereas *Anaplasmataceae* are either nonexistent (dos Santos et al., 2013; Skotarczak et al., 2006) or relatively common (14.3–49%) (Hornok et al., 2014; Ioannou et al., 2009; Machado et al., 2012). These patterns, however, are based on small samples from a variety of species. Furthermore, a majority of the birds examined were migratory, and little is known regarding differences between resident and migratory species in their potential to function as reservoirs for these pathogens.

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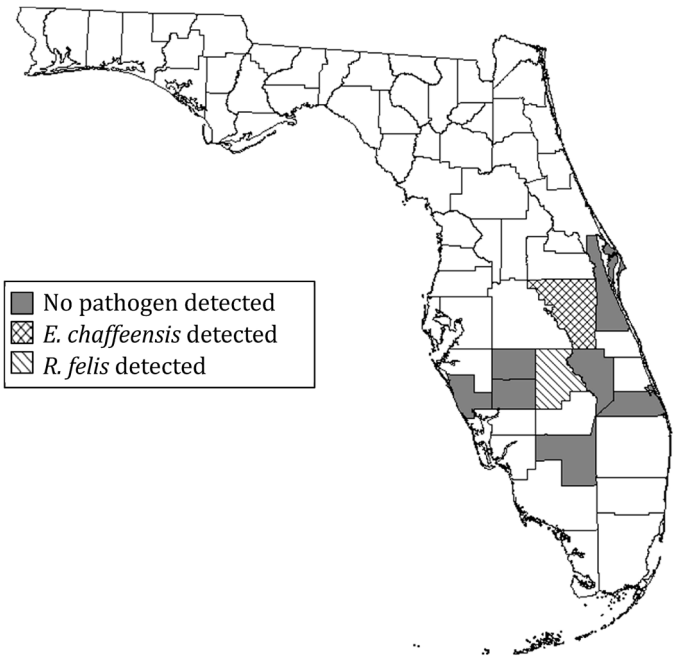

Fig. 1. *Map of Florida.* Counties with samples collected include: Brevard, DeSoto, Hardee, Hendry, Highlands, Martin, Okeechobee, Osceola, and Sarasota. *Ehrlichia chaffeensis* was detected in Osceola County (crossed fill). *Rickettsia felis* was detected in Highlands County (diagonal fill).

In Florida, the northern crested caracara (*C. cheriway*) is non-migratory and isolated from other caracara populations (Morrison and Dwyer, 2012). Caracaras spend extensive time on the ground primarily scavenging but also opportunistically hunting a variety of small mammals, birds, reptiles, amphibians, and invertebrates (Morrison and Dwyer, 2012). This population is known to harbor a variety of ectoparasites, including both mites (*Dubininia sp.*, *Hierocolichus* sp., *Ornitholnyssus bursa;* M. Spalding, J. Mertins, and J. Morrison unpubl. data) and chewing lice (*Acutifrons mexicanus, Colphocephalum polybori, Laemobothrion maximum*, *Falcolipeurus josephi*; Forrester et al., 1995; Tandan and Dhanda, 1963), and infections by zoonotic arboviruses have been reported (Nemeth et al., 2009). However, little is still known regarding the role caracaras, or other resident bird populations, may play in the maintenance and transmission of vector-borne diseases. In this study, we examined the prevalence of the *Anaplasmataceae* (*Ehrlichia sp.* and *Anaplasma sp.*) and *Rickettsiaceae* in the blood of the northern crested caracara (*C. cheriway*) from this resident population in Florida, U.S.A. Our results are relevant to the understanding of how avian hosts contribute to the transmission of vector-borne diseases.

Materials and methods

We collected whole blood from 55 wild northern crested caracaras (*C. cheriway*) from Florida and from an additional six captive individuals originating from Texas (Fig. 1). Samples were collected over two different field seasons: January–September 2007 and February through April 2011. These sampling periods corresponded with known activity of a variety of ixodid tick species of all life stages in Florida, including the peak seasons for *Amblyomma americanum* (Cilek and Olson, 2000) and *Dermacentor variabilis* (McEnroe, 1979). We used local mammals opportunistically recovered from roadsides after being struck by vehicles to attract wild crested caracaras to a bal-chatri trap (Bub, 1991) modified to accommodate carrion bait. We collected 0.2–0.6 mL of whole blood from the ulnar vein of each bird, fitted each with leg bands to facilitate future identification in the wild, and then immediately released them at the capture location. All birds were examined for ticks prior to release. We stored each blood sample for up to 4 h on ice until the sample could be centrifuged prior to deposition in long-term frozen storage. All protocols were approved by the Virginia Tech Institutional Animal Care and Use Committee (permit # 10-011-FIW).

The separated blood was homogenized by vortexing prior to DNA extraction. We used a DNeasy Blood and Tissue Kit (Qiagen Inc.) to extract DNA following the manufacturer's recommendations. To detect the presence of rickettsiae we used a semi-nested PCR design (Kelly et al., 2005; Stothard, 1995) (Table 1) to amplify a ~434 bp fragment of the 17 kDa antigen gene. The 17-kd gene is specific to the genus Rickettsia and contains sufficient inter-specific variation for the individual detection of species (Anderson, 1990; Carmichael and Fuerst, 2010). To detect the presence of ehrlichiae and anaplasmae we used a nested PCR developed for the *groESL* gene (Sumner et al., 1997) (Table 1). All primary PCR reactions contained 2 μL DNA template, 0.5 μM forward and reverse primers, 1X buffer (USB Corporation, Cleveland, OH), 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.05% bovine serum albumin, and 0.5 U *Taq* polymerase (USB Corporation) in a final volume of 10 μL. The semi-nested and nested reactions were the same except we used 2 μL of the primary PCR reaction as the template, 1 U *Taq* polymerase, and a final volume of 20 μL. Amplification conditions were as follows: 95 °C for 10 min, 35 cycles of 95 °C for 30 s, 52/55 °C for 30 s (primary and nested reactions, respectively), 72 °C for 1 min, and a final extension step at 72 °C for 7 min.

In each PCR reaction we included a positive control DNA sample (a known *Rickettsia amblyommii*-positive tick and cultured *E. chaffeensis* Arkansas) and negative control sample consisting of sterile, reagent-grade water. We used separate laboratories for the extraction of DNA and the PCR reactions. We observed the results of the PCR reactions through electrophoresis on a 1% agarose gel stained with ethidium bromide. Positive reactions were cleaned using the ExoSAP-IT PCR Clean-up kit (USB) following manufacturer's recommendations and sequenced in both the forward and reverse directions on an Applied Biosystems 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). We removed primers from all sequences, inspected chromatograms for errors, and combined forward and reverse reads into a consensus sequence using SEQUENCHER v 5.0 (Gene Codes Corp., Ann Arbor, MI). Consensus sequences were compared against the non-redundant NCBI database using BLASTN (http://blast.ncbi.nlm.nih.gov/).

Results and discussion

We detected the presence of a rickettsia in only one individual caracara (1.64%) from Highlands County, Florida (Table 1, Fig. 1). The rickettsia amplified shared 100% sequence identity across the entire fragment length (371 bp) with two isolates of *Rickettsia felis* (CP000053 and AF195118). *R. felis* is a flea-borne rickettsia that has been implicated in a growing number of human rickettsioses (Parola et al., 2005). *R. felis* has been reported from hen fleas (*Echidnophaga gallinacean*) (Jiang et al., 2013; Leulmi et al., 2014), which are known to infest a range of avian and mammalian hosts, including northern crested caracaras from Mexico (Santos et al., 2011). *R. felis* has also been reported in ticks collected from a pelican (*Pelecanus occidentalis*) rookery in South Carolina, U.S.A. (Reeves et al., 2006), demonstrating further the potential association with birds. The low prevalence of rickettsiae we observed is consistent with other reports from birds (Hornok et al., 2014; Ioannou et al., 2009).

For ehrlichiae, one individual caracara tested positive (1.64%), from Osceola County, Florida (Table 1, Fig. 1). The ehrlichia sequenced shared 100% sequence identity across the entire fragment length (490 bp) with a number of *E. chaffeensis* isolates (e.g.

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Table 1
Summary of the PCR primers used and bacteria amplified from northern crested caracaras.

Target genus	Genes	Primary primers		Nested primers		Number of positives			Blast results (accession)
						Florida $n=55$	Texas $n=6$	Total $n=61$	
Ehrlichia/Anaplasma[a]	groESL	Aphy HS1 Aphy HS6	TGGGCTGGTA(A/C)TGAAAT CCICCIGGIACIA(C/T)ACCTTC	Aphy HS43 Aphy HS45	AT(A/T)GC(A/T)AA(G/A)GAAGCATAGTC ACTTCACG(C/T)(C/T)TCATAGAC	1	0	1	*E. chaffeensis* (CP000236)
Rickettsia[b]	17 kDa	17-kD 5′ 17-kD 3′	GCTTTACAAAATTCTAAAAACCATATA CTTGCCATTGTCCRTCAGGTTG	17- kD 5′ 17-kD 3′ nest	GCTTTACAAAATTCTAAAAACCATATA TCACCGCAATATTGACC	1	0	1	*R. felis* (CP000053)

[a] Sumner et al. (1997).
[b] Stothard (1995) and Kelly et al. (2005).

CP007480.1, CP007476.1, L10917.1). This is first study to report an *E. chaffeensis* infection in caracaras (*Caracara* sp.), although other bacteria of the family *Anaplasmataceae* (*A. phagocytophilum*) have been identified from a southern crested caracara (*Caracara plancus*) in Brazil (Machado et al., 2012). We did not detect the presence of *A. phagocytophilum*, which was also absent from a large investigation of a variety of bird species from Brazil (dos Santos et al., 2013). In general, the prevalence of anaplasmae in birds seems to be locality- and/or species-specific and varies considerably (0–49%) (dos Santos et al., 2013; Hornok et al., 2014; Ioannou et al., 2009; Machado et al., 2012; Skotarczak et al., 2006). Although our PCR reactions did not include a positive control for the amplification of anaplasmae the primer set used was designed with specific degenerate nucleotides to match multiple species (Sumner et al., 1997) and is commonly used to detect these species (e.g., Massung and Slater, 2003; de Sousa et al., 2012; Welc-Falęciak et al., 2013). However, we cannot exclude the possibility our reactions failed to amplify anaplasmae and thus our results may underestimate the true prevalence.

None of the captive caracaras from Texas was positive for either primer set. This result is not surprising considering the very low sample size ($n=6$), the low prevalence we observed in the wild population from Florida, and that reported in the other studies discussed above. It is possible the captive population had been exposed to various treatments such as acaricides or antibiotics that would reduce our likelihood of detection. However, these birds were kept in outdoor cages and through the environment or interactions with wild individuals temporarily held at the facility are exposed to potential parasites.

It is well accepted that migratory bird populations contribute to the circulation of vectors of zoonotic pathogens (Alekseev et al., 2001; Björsdorff et al., 2001; Hildebrandt et al., 2010). However, the degree to which resident bird populations serve as reservoirs is much less clear. Reservoirs become especially important for agents like *E. chaffeensis*, which lack transovarial transmission in their primary tick host (Long et al., 2003) and require reservoirs for transmission. The primary vector of *E. chaffeensis*, *A. americanum*, is commonly found on wild turkeys (*Meleagris gallopavo*), and the increasing abundance of wild turkeys across North America may be a contributing factor in the rising importance of ehrlichiosis as a public health threat (Paddock and Childs, 2003). Like turkeys, northern crested caracaras forage extensively on the ground, often in close proximity to other carrion feeding birds (Dwyer, 2014; Morrison and Dwyer, 2012) thus providing a high potential for encountering vectors like *A. americanum*. We did not observe any ticks on the caracaras collected, yet our results suggest that caracaras may, at least occasionally, be parasitized by a vector of *E. chaffeensis* like *A. americanum*. The *E. chaffeensis*-positive individual was collected in April 2007, which coincides with near-peak season (May) for adult *A. americanum* in Florida (Cilek and Olson, 2000). The ecology of *R. felis*, on the other hand, remains relatively unclear. It is stably transmitted in laboratory colonies of cat fleas (*Ctenocephalides felis*), its principal vector (Reif and Macaluso, 2009), yet its maintenance in nature is best explained by horizontal transmission through co-feeding with both intra- and interspecific arthropod vectors (Brown et al., 2015). It is plausible our detection of *R. felis* rickettsemia in caracaras is a by-product of the co-feeding process between known arthropod parasites (Tandan and Dhanda, 1963; Forrester et al., 1995; Santos et al., 2011) and does not represent a disseminated infection (Labruna and Walker, 2014).

Conclusions

This study provides one of the most comprehensive surveys of Anaplasmataceae and Rickettsiaceae in a single bird species and is the first to examine the northern crested caracara as a

potential reservoir host. Our results suggest that *C. cheriway* are rarely infected with the zoonoses we investigated and likely don't function as a significant reservoir. However, it is possible that the prevalence of infection may vary considerably across seasons and/or years, and more extensive surveys of this kind are necessary to substantiate these findings. Furthermore, little is known regarding whether the bacteria we detected represent inadvertent transmissions or acute, enzootic infections. Future studies examining the arthropod parasites of caracaras in addition to other resident bird populations may provide additional insight into the natural epidemiology of these and other zoonotic infections.

Author disclosure statement

The authors declare that no competing financial interests exist.

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