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Short communication

The prevalence of rickettsial and ehrlichial organisms in *Amblyomma americanum* ticks collected from Ohio and surrounding areas between 2000 and 2010

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ABSTRACT

The lone star tick, *Amblyomma americanum*, feeds upon a variety of hosts and is a known vector of several human pathogens. In Ohio, populations of *A. americanum* have been expanding their range and increasing in abundance and distribution, thereby elevating the public health concerns regarding bites from this species. We used a set of PCR assays to detect the presence of ehrlichial and rickettsial species in *A. americanum* ticks submitted to the Ohio Department of Health Zoonotic Disease Program over an 11-year period (2000–2010). We did not detect the presence of known pathogens *Rickettsia rickettsii* or *Ehrlichia chaffeensis*, but we did identify the presence of two other bacterial species: '*Candidatus* Rickettsia amblyommii', and *Ehrlichia* sp. Panola Mountain. '*Candidatus* R. amblyommii' was the most common species identified (30.2%), whereas the ehrlichiae was quite rare (0.6%). With growing evidence implicating both '*Candidatus* Rickettsia amblyommii' and *Ehrlichia* sp. Panola Mountain in mild to moderate human disease, our results support the importance of continued monitoring of *A. americanum* ticks for the presence of potential pathogens.

Introduction

Amblyomma americanum (L.) (Acari: Ixodidae), commonly known as the lone star tick, is widely distributed throughout much of the central and eastern United States. During all life stages it feeds upon a variety of ground-nesting birds, reptiles, and mammals, including humans (Merten and Durden, 2000; Paddock and Yabsley, 2007). Historically, *A. americanum* was considered a nuisance species and not a threat to human health (Schulze and Bosler, 1996). However, public health concerns regarding *A. americanum* are rising in response to their increasing abundance, expanding distribution, and potential to transmit a variety of infectious diseases (e.g. rickettsiosis, ehrlichiosis, tularemia, southern tick-associated rash illness, Lyme borreliosis, and Heartland virus) to humans and domestic species (Childs and Paddock, 2003; Paddock and Yabsley, 2007; Clark et al., 2013; Savage et al., 2013).

Once considered the periphery of *A. americanum*'s range, Ohio is now home to many established populations of *A. americanum*. The expansion of *A. americanum* into Ohio is consistent with the first confirmed report of ehrlichiosis in the state in 2006. Since the index case, ehrlichiosis has remained relatively uncommon, but is likely underreported (Ohio Department of Health Zoonotic Disease Program, Mary Daniels, personal communication). Despite the spread of *A. americanum* into Ohio, little is known regarding the prevalence of potential disease-causing organisms transmitted by this tick in Ohio and the threats posed to humans. To date, the most extensive studies of *A. americanum* either did not include Ohio (Mixson et al., 2006; Apperson et al., 2008; Stromdahl et al., 2008; Jiang et al., 2010; Smith et al., 2010) or examined ≤22 ticks from Ohio (Kelly et al., 2005; Loftis et al., 2008). To address these concerns, we assessed the prevalence of ehrlichial and rickettsial species in *A. americanum* collected primarily from Ohio and other nearby states over an 11-year period (2000–2010). Our lab previously reported the prevalence of rickettsial species in 21 Ohio *A. americanum* in

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Table 1
Summary of the screens for *Rickettsia* sp. and *Ehrlichia* sp. bacteria in *Amblyomma americanum* ticks from Ohio and other (non-Ohio) states. The dashes indicate no ticks were made available for testing for that year.

Target	*Amblyomma americanum* ticks	2000	2001	2002	2003	2004	2005	2006	2007[a]	2008	2009	2010	Total
Rickettsiae in Ohio ticks	# Tested	20	4	–	12	–	–	64	29	28	77	74	308
	# Positive	7	0	–	4	–	–	10	11	11	24	26	93
	Prevalence	0.350	0.00	–	0.333	–	–	0.156	0.379	0.393	0.312	0.351	0.302
Rickettsiae in non-Ohio ticks	# Tested	6	4	–	6	–	–	–	–	–	11	–	27
	# Positive	1	2	–	5	–	–	–	–	–	2	–	10
	Prevalence	0.167	0.500	–	0.833	–	–	–	–	–	0.182	–	0.370
Ehrlichiae in Ohio ticks	# Tested	16	4	–	46	–	–	64	30	17	75	75	327
	# Positive	0	1	–	0	–	–	0	1	0	0	0	2
	Prevalence	0.00	0.250	–	0.00	–	–	0.00	0.033	0.00	0.00	0.00	0.006
Ehrlichiae in non-Ohio ticks	# Tested	6	4	–	20	–	–	–	–	–	11	–	41
	# Positive	0	1	–	0	–	–	–	–	–	0	–	1
	Prevalence	0.00	0.250	–	0.00	–	–	–	–	–	0.00	–	0.024

[a] Field-collected ticks (see Materials and methods).

2003 (Kelly et al., 2005), and this study included those results for completeness.

Materials and methods

We obtained a portion of *A. americanum* received by the Ohio Department of Health Zoonotic Disease Program (ODH-ZDP) between the years 2000 and 2010. For over 30 years, the ODH-ZDP has provided tick identification and testing to Ohio citizens. The primary goal is to provide education regarding tick-borne disease and to passively monitor tick populations and pathogens. At the time, *A. americanum* was considered uncommon and the program only monitored American dog ticks, *Dermacentor variabilis*, for the presence of *Rickettsia rickettsii* (the etiological agent of Rocky Mountain spotted fever). Ticks were routinely submitted to the program from veterinarians, clinicians or local health departments across the state. Occasionally the program received ticks reported to originate from areas outside of Ohio (e.g. Ohio residents that have recently traveled out of state), which we used to compare with those ticks from Ohio. No ticks were made available for testing in years 2002, 2004–2005, and 2007. In 2007 we collected additional questing *A. americanum* from multiple locations in Lawrence and Jackson Counties, Ohio, using a flagging method described elsewhere (Whitlock et al., 2000). All ticks were surface sterilized using 70% ethanol, rinsed with distilled water, and dissected in extraction buffers. We purified genomic DNA using either the Isoquick Extraction Kit (Orca Research, Bothell, WA, USA) or the DNeasy Tissue Kit (Qiagen Inc., Valencia, CA, USA) according to manufacturers' protocols.

To detect spotted fever group rickettsiae we amplified a fragment of the conserved 17 kDa antigen gene using a semi-nested primer set previously developed in our laboratory (Stothard, 1995; Kelly et al., 2005). We screened for the presence of ehrlichiae using a nested PCR for a portion of the *Ehrlichia sp*. Panola Mountain *gltA* gene (Loftis et al., 2008). Each PCR reaction included a positive control (cultured *Rickettsia conorii* VR-141 or *E. chaffeensis* Arkansas DNA) and negative control containing deionized water that was subsequently reamplified in the semi-nested and nested PCR reactions. Successful amplification was observed using a 1.0% agarose gel stained with ethidium bromide. PCR fragments were prepared for sequencing using either the Qiaquick PCR Purification Kit (Qiagen Inc.) or the ExoSAP-IT PCR Clean-up kit (USB Corporation, Cleveland, OH) following manufacturer's recommendations. We sequenced amplified fragments on an Applied Biosystems 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). All sequences were assembled and visually inspected for errors using Sequencher 5.0 (Gene Codes Corp., Ann Arbor, MI). We identified the species by comparing our sequences to the non-redundant NCBI database (http://www.ncbi.nlm.nih.gov/) using BLASTN (http://blast.ncbi.nlm.nih.gov/) with a threshold match identity of at least 95% and an expect value (e-value) less than 10^{-30}.

Results

Of the 387 ticks examined, 318 (82.2%) were screened with both sets of primers. An additional 18 (4.7%) and 51 (13.2%) ticks were analyzed with only the rickettsia or ehrlichia primer sets, respectively, due to lack of available materials.

Within Ohio we screened a total of 308 ticks for rickettsiae using the 17-kDa antigen gene. In addition, a group of 27 ticks reported from areas outside of Ohio was examined for comparative purposes (Table 1). A total of 93 ticks from Ohio were found to be positive for rickettsiae (30.2%). This was slightly less than the prevalence of rickettsiae in non-Ohio ticks (37.0%). Of the Ohio positive samples, all were identified as '*Candidatus* R. amblyommii', whereas a single sample from Arkansas was identified as *Rickettsia bellii* (reported previously by Kelly et al., 2005). The yearly prevalence of rickettsia-infected ticks in Ohio ranged between 15.6% and 39.3%, excluding the year 2001 (which contained no positive ticks in a sample size of only four) (Table 1).

Within Ohio, we assessed ticks submitted from 50 of 88 (56.8%) counties for rickettsial agents (Fig. 1A). We detected *Candidatus* R. amblyommii-positive ticks in 29 (58.0%) of the counties tested. Of the counties where at least 10 ticks were tested (Butler, Gallia, Montgomery, Ross, Pike, Lawrence, Jackson, and Scioto Counties), the prevalence of *Candidatus* R. amblyommii-positive ticks ranged between 16.7% (Butler County) and 35.4% (Scioto County). An additional 3 of 6 ticks from Ohio were *Candidatus* R. amblyommii-positive but lacked county information.

A nested PCR assay for the conserved *gltA* gene was used to screen for the presence of ehrlichiae (Table 1). We identified the presence of ehrlichiae in 2 of 327 (0.6%) of Ohio ticks screened. Only 1 of 41 (2.4%) ticks was positive from the comparison group, with a single positive tick from Virginia. Within Ohio, the two positive ticks were from Lawrence and Scioto Counties (Fig. 1B). All positive samples were identified as *Ehrlichia* sp. Panola Mountain, and we did not detect any *E. chaffeensis*-infected ticks. In the samples of most years, we did not detect any positive ticks. In Ohio the *Ehrlichia* sp. Panola Mountain-positive ticks were found in samples from 2001 and 2007 (Table 1).

Discussion

Multi-state studies of the prevalence of infected *A. americanum* has also been obtained for New York, New Jersey, Georgia, Florida, Oklahoma and Iowa (Mixson et al., 2006) and for a large number of

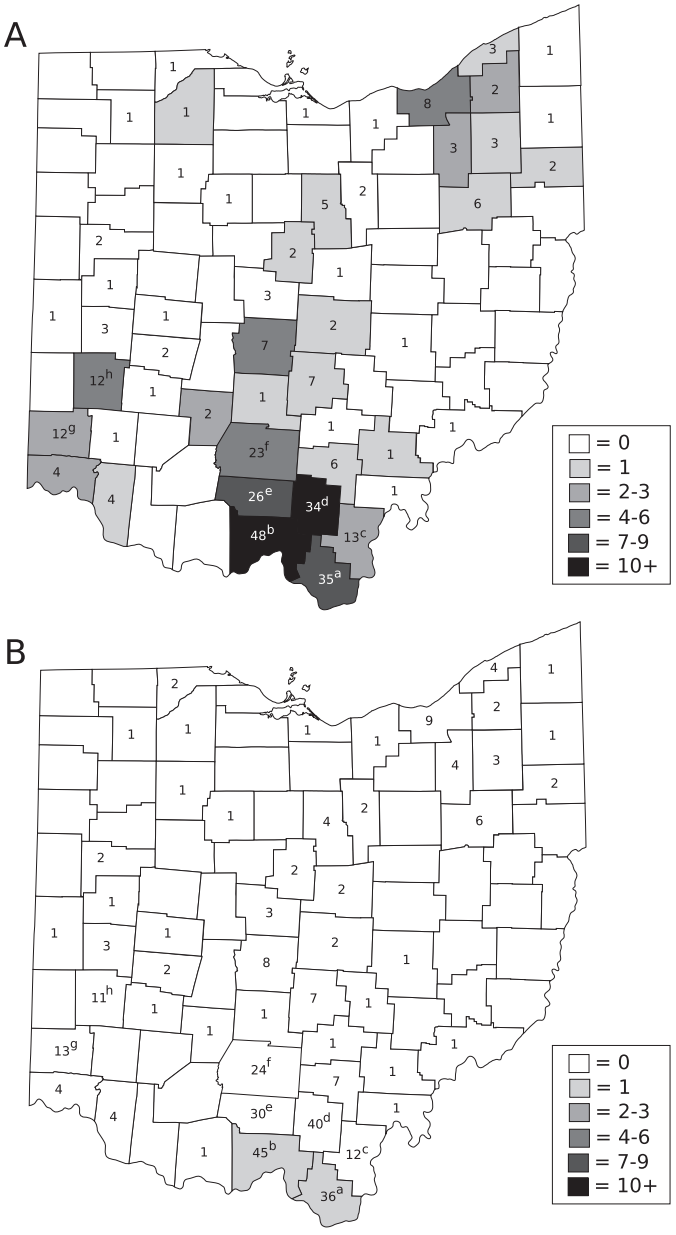

Fig. 1. County maps of Ohio. The number inside each county represents the number of ticks tested that were from that county. The shading indicates the number of (A) *Rickettsia*-positive and (B) *Ehrlichia*-positive ticks according to the legend given. Six and 11 *A. americanum* (including 3 *Rickettsia*-positive) samples are not shown in (A) and (B), respectively, because they lacked county information. Superscript letters represent those counties mentioned in the text*. A map of Ohio's counties with names is available at http://quickfacts.census.gov/qfd/maps/ohio_map.html. Maps were recreated under a license to distribute freely available from Wikipedia Commons (http://commons.wikimedia.org/wiki/Main_Page). *[a] Lawrence County, [b] Scioto County, [c] Gallia County, [d] Jackson County, [e] Pike County, [f] Ross County, [g] Butler County, [h] Montgomery County.

Department of Defense personnel and dependents in many states (Stromdahl et al., 2011). Single-state studies have also been performed in Texas (Williamson et al., 2010), North Carolina (Mixson et al., 2006; Apperson et al., 2008; Smith et al., 2010), Kansas (Berrada et al., 2011), Kentucky (Fritzen et al., 2011), and Tennessee (Moncayo et al., 2010). None of these studies included Ohio.

Previous studies have documented the prevalence of '*Candidatus* R. amblyommii' infection in *A. americanum* to range between zero and ~90%, depending upon location and year, with typical rates approaching 40–60% (Mixson et al., 2006; Apperson et al., 2008; Stromdahl et al., 2008; Jiang et al., 2010; Smith et al., 2010). In Ohio, the overall prevalence of '*Candidatus* R. amblyommii' was between 15.6% and 39.3%, similar to the previously published ranges. The highest rates of infection were in the southern counties of the state, congruent with a northward expansion of tick populations and known densities (Tick-borne Diseases Ohio Summary, 2011). In Kentucky, where *A. americanum* has existed for many years in higher densities, the frequency of *Candidatus* R. amblyommii-positive ticks (28.7%) was very similar to that found in our study, whereas ehrlichia-positive ticks were more prevalent (9.2%) (Fritzen et al., 2011). Within Ohio ticks we did not detect any instance of infection with *R. bellii*, although a single *A. americanum* from Arkansas was infected (reported previously by our laboratory in Kelly et al., 2005). We also failed to detect any infection with *R. rickettsii* in our samples. *R. rickettsii* is the agent of Rocky Mountain spotted fever and has been detected in some other surveys of *A. americanum*, and is known to occur in Ohio and be present in Ohio *D. variabilis* ticks (Pretzman et al., 1990; Fuerst et al., 1990). We also did not detect any evidence of other spotted fever group forms, especially *Rickettsia montanensis*, which has been reported in *D. variabilis* from Ohio. In these earlier studies, *R. bellii*, *R. rickettsii* and *R. montanensis* were observed in about 3%, 0.06% and 0.08% of *D. variabilis*, respectively (Pretzman et al., 1990; Fuerst et al., 1990).

We also screened for the presence of *Ehrlichia* sp. Panola Mountain and *E. chaffeensis*. Although the primers used to amplify a fragment of the *gltA* gene (Loftis et al., 2008) were developed for the Panola Mountain ehrlichia, we used DNA from *E. chaffeensis* as a positive control to verify the ability of our primers to successfully amplify the gene of this ehrlichial species. We found the occurrence of *Ehrlichia* sp. Panola Mountain to be quite rare (0.6% in Ohio). This result is similar to that reported across the range of *A. americanum*, but substantially lower than that reported previously from 22 Ohio ticks (27%, Loftis et al., 2008). The much lower prevalence of *Ehrlichia* sp. Panola Mountain in this study could be function of the false positive rate and sensitivity of the PCR reaction, as successful amplification can be influenced by a variety of reaction conditions (Apfalter et al., 2005). Nevertheless, future work employing quantitative real-time PCR for the 16S rRNA gene, which has been shown to be as sensitive as nested PCR for the detection of ehrlichia (Loftis et al., 2003), may be necessary to substantiate our findings. We did not detect the presence of *E. chaffeensis*, although human cases have been reported from Ohio, including three cases in 2012 from southern Ohio counties (Mary Daniels, personal communication; MMWR, 2013). Furthermore, *E. chaffeensis*-reactive antibodies were identified in 11/327 (3.4%) of white-tailed deer tested from Ohio (Irving et al., 2000). All 11 of the positive deer samples were from southern Ohio counties, including two from Lawrence County. These results suggest the potential presence of *E. chaffeensis* in Ohio ticks. However, because cross-reactivity has been demonstrated between *Ehrlichia* sp. Panola Mountain and *E. chaffeensis* (Reeves et al., 2008), as well as between *Ehrlichia ewingii* and *E. chaffeensis* (Buller et al., 1999; Paddock et al., 2001), we cannot exclude the possibility that the reports of *E. chaffeensis* infections in humans or white-tailed deer may be different *Ehrlichia* species. Also, these cases may be the result of infections acquired from outside the state, or that other vectors of *E. chaffeensis*, like *D. variabilis* (Holden et al., 2003), are responsible.

Overall, the lone star tick can potentially transmit a variety of pathogens to humans, domestic animals, and wildlife (Paddock and Yabsley, 2007). As the distribution of *A. americanum* expands northward, we expect to see an increase in the number of reported cases of these diseases in Ohio. Despite the fact that the geographic locations reported for each tick by clinicians may not always represent the true origin of the tick, it appears the highest potential for transmission of these bacteria is in southern Ohio. Our data also suggest the transmission of either *Ehrlichia* sp. Panola Mountain or

E. chaffeensis to humans in Ohio from *A. americanum* is, thus far, rather uncommon, but mild rickettsiosis in humans caused by '*Candidatus* R. amblyommii' may present a threat (Sanchez et al., 1992; Dasch et al., 1993; Billeter et al., 2007). Unfortunately, due to budgetary restrictions, the tick and mosquito component of the ODH-ZDP was eliminated in 2013, and this may prevent new pathogens from being detected until human cases are diagnosed. Therefore, it is important for clinicians to assess a patient's potential exposure to ticks to consider the potential for tick-borne disease. Chapman et al. (2006) provide a useful resource for health care practitioners to use as a guide when diagnosing and treating tick-borne rickettsial diseases. Additionally, clinicians should be aware of programs, such as the ODH-ZDP, which are vital for monitoring the threats posed to human and animal health from vector-borne diseases.

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