Novel Spotted Fever Group Rickettsiae (SFGR) Infecting *Amblyomma americanum* Ticks in Ohio, USA

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OBJECTIVES

To determine the prevalence, species composition, and genetic diversity of tick-borne rickettsiae found in *Amblyomma americanum* ticks in Ohio.

BACKGROUND

Responding to cases of tick-borne Rocky Mountain spotted fever (RMSF), the Ohio Department of Health (ODH) Vector-Borne Disease Unit (VBDU) initiated a tick testing program in 1964. Health care professionals in Ohio and other states submitted ticks collected from pets or patients. Primarily, samples of the American dog tick (*Dermacentor variabilis*), the vector of *Rickettsia rickettsii*, were tested for rickettsiae. ODH-VBDU also frequently received lone star ticks (*Amblyomma americanum*), but, as less likely vectors, these were not tested for rickettsiae until recently.

In 1974 an unknown "non-pathogenic" rickettsiae, designated WB-8-2, was isolated from an *A. americanum* tick collected in Tennessee.[1] Subsequently, in 1985, a similar form (MO-1084) was isolated from a tick collected at Fort Leonard Wood, Missouri.[2] Analyses in our laboratory of the 16S rRNA gene and the 17-kDa cell surface antigen protein gene showed that these isolates carried identical genotypes for the genes and the name "*R.* amblyommii" sp. nov. was proposed.[3] This form has so far been found in *A. americanum*, but not in ticks from the genera *Dermacentor* or *Rhipicephalus*.

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Rickettsia bellii was isolated in 1966, and is usually associated with *Dermacentor* sp., and with some argasid ticks. To our knowledge, it has not been reported in *A. americanum.* In Ohio, *Dermacentor variabilis* is known to carry *R. rickettsii*, and *R. montanensis,* in addition to *R. bellii.* Since *D. variabilis* and *A. americanum* are likely to share mammalian hosts, *A. americanum* may serve as host for these other rickettsiae. Here, we seek to determine prevalence, species identification, and genetic diversity of nonpathogenic rickettsiae in *A. americanum* ticks collected primarily in Ohio.

MATERIALS AND METHODS

Specimens of *A. americanum* were sent directly from veterinarians, clinicians, or local health departments to ODH-VBDU from various locales as part of the tick screening program. *A. americanum* ticks, mostly attached to humans and collected primarily in Ohio in 2003 submitted to ODH-VBDU were subsequently provided to our laboratory. Frozen ticks (–20°C) were surface sterilized in 70% ethanol, washed in distilled water, and chopped in extraction buffers using a sterile needle to expose internal tissues. Genomic DNA was extracted using the PrepMan Ultra extraction solution (Applied Biosystems, Foster City, CA, USA) or a combination of the Isoquick Extraction Kit (Orca Research, Bothell, WA, USA) and DNeasy Tissue Kit (Quiagen Sciences, Valencia, CA, USA). Semi-nested PCR was done in a Whatman-Biometra thermal cycler (Biometra Biomedicine Analytik, Goettigen, Germany) with paired genus-specific oligonucleotide primers (17kD-5′ GCTTTACAAAA TTCTAAAAACCATATA; 17kD-3′ CTTGCCATTGTCCRTCAGGTTG; and 17kD-3′ nest TCACGGCAATATTGACC). These primer sequences were designed based upon work done in our laboratory.[3] Distilled water was used as a negative control for the primary PCR, a portion of which was reamplified as the semi-nested PCR negative control. Amplicons derived from semi-nested PCR were approximately 434 bp. Conditions for both primary and semi-nested PCR were as follows: 10 min at 95°C, 35 cycles of 1 min at 95°C, 1 min at 52°C, 1 min at 72°C, 15 min extension at 72°C). Direct sequencing of the PCR product was done using the Big Dye Terminator Sequencing Kit, using sequencing primer 17kD-5′seq-GGTTCTCAATTYGG and the ABI 310 system (Applied Biosystems).

RESULTS

A subset of *A. americanum* ticks obtained during the 2003 tick screening program of the ODH-VBDU were surveyed for the presence of rickettsiae using PCR and DNA sequencing. Prior to 2003, *Amblyomma* ticks were not routinely examined for the presence of tick-borne pathogens. Nine of 21 *Amblyomma* ticks examined (43%) showed evidence of carrying rickettsiae. Of the nine rickettsia-positive ticks, eight (89%) were carrying strains identified by sequence analysis as *R. amblyommii* sp. nov. A single tick was found to carry a strain identified as *R. bellii.* Geographically, the ticks that were studied in this subset of material from the ODH-VBDU tick screen represented material primarily from central and south Ohio. These are regions

that have been shown to have relatively high incidence of RMSF and to have a high occurrence of *D. variabilis* ticks found to be rickettsiae-positive.

DISCUSSION

Results for the molecular survey of *A. americanum* from the 2003 ODH-VBDU tick screening program showed a high prevalence of rickettsiae-positive ticks (43%). This is much higher than seen in screens of *Dermacentor,* where rickettsiae-positive ticks, assessed by hemolymph staining and IFA screening were consistently observed in frequencies ~ 4–5% over a number of years.

Rickettsiae-positive *Amblyomma* ticks are identified in the same parts of Ohio where rickettsiae-positive *Dermacentor* ticks were observed. However, the distribution of the forms of *Rickettsia* found in *Amblyomma* compared to those found in *Dermacentor* was quite different. In *Amblyomma* from Ohio, almost all rickettsiae were identified, using molecular methods, as *R.* amblyommii sp. nov. A single tick carried a strain of *R. bellii*. In contrast, using molecular methods to identify rickettsial forms carried by *D. variabilis* ticks in Ohio from earlier years, we found that the most frequent form was *R. bellii* (82% of isolates identified using molecular screens in our laboratory). In addition, two Spotted Fever Group forms, *R. montanensis* (12.5% of isolates) and *R. rickettsii* (5.5% of isolates) are also identified in *D. variabilis*. No isolates of *R. amblyommii* sp. nov. were observed in *D. variabilis*. It appears that the two tick groups do not easily exchange rickettsiae through intermediate mammalian hosts.

A. americanum is a vector for ehrlichial pathogens. The current study has not assessed the presence of any *Ehrlichia* in these ticks. Occurrence/co-occurrence of rickettsiae and ehrlichiae within *A. americanum* should be a subject for further and future investigation.

CONCLUSIONS

We found a high rate of occurrence of *R. amblyommii* sp. nov. in *Amblyomma* ticks from Ohio. The rickettsial infection rate in *A. americanum* from Ohio is higher than in *Dermacentor.* Before 2004, ODH-VBDU did not screen *A. americanum* for rickettsiae. Now, with evidence of tick-borne rickettsial and ehrlichial pathogens in *A. americanum* in the U.S., all ticks are individually tested.

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