EHRlichia CHAFFESENSIS IN MISSOURI Ticks

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Abstract. A nested polymerase chain reaction specific for Ehrlichia chaffeensis was used to attempt to amplify DNA from extracts of 100 individual ticks collected from 13 counties in central Missouri. Seventeen of 59 Amblyomma americanum and six of 41 Dermacentor variabilis ticks exhibited the characteristic 389-basepair product. This supports the hypothesis that these tick species may be vectors of human monocytic ehrlichiosis.

Human monocytic ehrlichiosis is a nonspecific febrile illness first described in 1987.1 Based on epidemiologic and polymerase chain reaction (PCR) studies, the etiologic agent, Ehrlichia chaffeensis, is believed to be transmitted predominantly by the Lone Star tick, Amblyomma americanum.2-3 Missouri is one of the leading states for reported cases of human monocytic ehrlichiosis.

The PCR has been used to demonstrate the presence of the 16S RNA gene sequence of E. chaffeensis in extracts of pooled ticks and in individual ticks.2,3 The former method used DNA extracted from a pool of ticks to potentially increase target DNA sequences, while the latter was felt to lack sensitivity.

A nested PCR greatly enhances sensitivity of detection of target nucleic acid sequences.3 This method has been used to increase the sensitivity for detection of E. chaffeensis in the blood of white-tailed deer.6 We now report the results of applying an E. chaffeensis-specific nested PCR technique to 100 individual ticks of the species A. americanum and Dermacentor variabilis collected from 13 counties in Missouri.

MATERIALS AND METHODS

Ticks were collected during the spring and summer of 1995 from 13 different counties in Missouri (Figure 1). Sources of the ticks included vegetation, domestic animals, and humans. The species, sex, and stage of development of each tick were identified by an entomologist; the sex of nymphal ticks could not be determined by visual inspection.

DNA was extracted from ticks by a modification of a method previously described.4 Briefly, individual ticks were placed in 200 μl of water, crushed, and boiled for 10 min. This material was diluted 1:2 in 10 mM Tris, pH 8.0, 10 mM NaCl and lysed in the presence of 1.0% sodium dodecyl sulfate and 100 ng of proteinase K/ml for 2 hr at 50°C. The lysed suspension was extracted twice with an equal volume of phenol-chloroform. A 1:10 dilution of 3 M sodium acetate, pH 5.5, and a 1:1,000 dilution of 20 mg/ml of glycogen (Boehringer Mannheim, Indianapolis, IN) were added to the extracted aqueous phase. The DNA was precipitated with cold ethanol, washed once with 70% ethanol, dried, and resuspended in 40 μl of water.

The DNA templates from individual ticks were examined by PCR by a modification of a method previously described.6 For the initial amplification, 10 μl of each template sample was amplified in a 60-μl reaction mixture containing the primers ECB (5'-CGTATTACCGGCTGCTGGCA-3') and EG1 (5'-CTCAGAAGCTGCTGCGG-3') and GeneAmp reagents (Perkin-Elmer Cetus, Norwalk, CT) at concentrations described in the GeneAmp protocol. ECB is part of the primer set of ECB and ECC originally described by Dawson and others known to amplify all known Ehrlichia species.4 The primer EG1, constructed previously by our laboratory to serve as a universal primer for Ehrlichia species, is a modification of the originally described primer ECC.6 The positive control was E. chaffeensis DNA isolated from infected Vero cells, and the negative control was water instead of the tick sample. Vero cells were infected with E. chaffeensis by a method previously described.7 The infected Vero cells were maintained in a separate laboratory, and DNA was extracted from them in a similar manner as described above. The following temperature profile was run for 30 cycles: 1 min at 94°C, 2 min at 60°C, and 1 min at 72°C.

For the nested PCR, 10 μl of each initial reaction product was amplified in a second 60-μl reaction mixture with E. chaffeensis-specific primers HE1 (5'-CAATGCCTATAAATTCTTATAAT-3') and HE3 (5'-TATAAGTCAGTCATTATCTTCCCT-3') as previously described.4 Amplification products were visualized by electrophoresis in a 1% agarose gel stained with ethidium bromide.

RESULTS

One hundred individual ticks were collected and characterized. This collection consisted of 59 A. americanum and 41 D. variabilis ticks. Fifty were females, 39 were males, and 11 were nymphs. Sixty-three of the ticks were from two counties (Boone and Cooper) with the remainder from 11 other counties in central Missouri (Figure 1).

The DNA extracts from 23 of the 100 ticks were positive after nested amplification with initial primers ECB and EG1 and subsequent primers HE1 and HE3, demonstrating the presence of the 389-basepair band characteristic of E. chaffeensis (Figure 2). Seventeen (29%) of the 59 A. americanum and six (15%) of the 41 D. variabilis ticks were positive. Also, 11 were female (eight A. americanum and three D. variabilis), six were male (three A. americanum and three D. variabilis), and six were nymphs (all A. americanum). These positive ticks were collected from vegetation (11 A. americanum and four D. variabilis), a dog (five A. americanum and two D. variabilis), and a human (one A. americanum). Neither the dog nor the human was reported to be ill after tick collection. Fifteen of the positive ticks came from Boone and Cooper.
Fig. 1. The 13 counties in Missouri where the 100 ticks were collected. Numerators in each county represent the number of ticks positive for *Ehrlichia chaffeensis* DNA by a nested polymerase chain reaction. Denominators represent the number of ticks collected in each county.

Fig. 2. UV-illuminated agarose gel showing the 389-basepair (bp) polymerase chain reaction product specific for *Ehrlichia chaffeensis* from representative samples. Lane m, mass marker; +, positive control (see Materials and Methods); –, negative control (see Materials and Methods); 1, *Amblyomma americanum* nymph; 2, *Dermacentor variabilis* nymph; 3, *A. americanum* adult; 4, *D. variabilis* adult.

DISCUSSION

Human monocytic ehrlichiosis is endemic in Missouri. These results demonstrate the presence of *E. chaffeensis* DNA in individual ticks collected in various counties in Missouri. To our knowledge, this is the first report of the detection of *E. chaffeensis* DNA in individual nymphal *A. americanum* ticks. The nested PCR has been previously used to demonstrate *E. chaffeensis* DNA in six individual adult *A. americanum* ticks.8

Anderson and others demonstrated *E. chaffeensis* DNA by PCR in one *D. variabilis* tick that was also positive by direct immunofluorescence for *Ehrlichia* species.4 Three *A. americanum* ticks that were positive by immunofluorescence for *Ehrlichia* were PCR negative. Anderson and others further demonstrated the presence of *E. chaffeensis* DNA by PCR in DNA extracts of pools of adult *A. americanum* ticks.7 No amplification products were seen in pools of nymphal *A. americanum* ticks or pools of *D. variabilis* and *Ixodes scapularis* ticks. Dawson and others aped the nested PCR to detect *E. chaffeensis* DNA in the blood of white-tailed deer.4 Nested amplification was required because of the low sensitivity of the initial reaction.

Our laboratory has applied a modification of the nested PCR technique of Dawson and others to demonstrate the presence of *E. chaffeensis* DNA in ticks. These results show the presence of *E. chaffeensis* DNA in individual adult ticks of two species and in individual nymphs of *A. americanum*, supporting the hypothesis that *A. americanum* is a vector of human monocytic ehrlichiosis. Our results suggest that *D. variabilis* may also play a role in the transmission of *E. chaffeensis*. While the number of ticks examined from some counties was small, our results suggest that parasitized ticks are widespread in central Missouri.

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