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Raccoons (*Procyon lotor*), but not rodents, are natural and experimental hosts for an ehrlichial organism related to "*Candidatus* Neoehrlichia mikurensis"

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Abstract

"*Candidatus* Neoehrlichia mikurensis" has been reported from a variety of rodent and *Ixodes* tick species in Europe and Asia. Recently, an ehrlichial organism closely related to "*Candidatus* Neoehrlichia mikurensis" was cultured from a raccoon (*Procyon lotor*) from Georgia, USA. To determine prevalence and distribution, we conducted a molecular survey of free-ranging raccoons ($n = 197$) from 10 populations in 3 states and found that infections were common in tick-infested populations (50–94%). In an effort to determine the host range of this organism, 10 species of rodents ($n = 137$) trapped in 3 areas where positive raccoons had been detected were tested; all were negative. In addition, captive bred raccoons and several common laboratory animals (mice, rats, and rabbits) were inoculated with the raccoon ehrlichial isolate (strain RAC413). Raccoons became infected with the culture isolate but all other hosts were refractory to infection. The 16S rRNA gene sequence (1379bp) of the RAC413 isolate was most similar (98.4–98.8%) to members of the "*Candidatus* Neoehrlichia mikurensis" group and phylogenetic analysis confirmed this organism was related to, but distinct from, "*Candidatus* Neoehrlichia mikurensis". Based on the molecular and natural history uniqueness of this organism from raccoons, we propose that this represents a novel species in the "*Candidatus* Neoehrlichia" group of ehrlichial organisms.

Keywords: Raccoon; Neoehrlichia; Rodents; Candidatus; Amblyomma; Ixodex; Dermacentor

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1. Introduction

''*Candidatus* Neoehrlichia mikurensis'' (Family *Anaplasmataceae*) is a group of bacteria detected in several species of ticks and rodents from Europe and Asia (Schouls et al., 1999; Brouqui et al., 2003; Pan et al., 2003; Kawahara et al., 2004; Beninati et al., 2006; Rar et al., 2008). Members of the ''*Candidatus* Neoehrlichia mikurensis'' group are distinguished from other genera based on sequence analysis of 16SrRNA, citrate synthase (*gltA*), and *groESL* genes (Brouqui et al., 2003; Kawahara et al., 2004). Sequences of ''*Candidatus* Neoehrlichia mikurensis'' were first detected in *Ixodes ricinus* ticks in the Netherlands and were described as an *Ehrlichia*-like sp. Shotti variant (Schouls et al., 1999) and subsequently have been detected in *I. ricinus* in Russia (Alekseev et al., 2001) and Italy (called ''*Candidatus* Ehrlichia walkerii'') (Brouqui et al., 2003), *I. persulcatus* in Russia (Rar et al., 2008), and *I. ovatus* from Japan (Pan et al., 2003; Kawahara et al., 2004).

To date, the only known vertebrate hosts of ''*Candidatus* Neoehrlichia mikurensis'' are various species of rodents in Japan, China, and Italy. Natural infections have been reported from rats (*Rattus norvegicus*) from China and Japan, small Japanese field mice (*Apodemus argenteus*), large Japanese field mice (*A. speciosus*), gray red-backed voles (*Clethrionomys rufocanus bedfordiae*) and Pratt's voles (*Eothenomys smithii*) in Japan, an East European field vole (*Microtus rossiaemeridionalis*) from Russia (Rar et al., 2008), and a bank vole (*C. glareolus*) from Italy (Pan et al., 2003; Kawahara et al., 2004; Beninati et al., 2006; Naitou et al., 2006; Tabara et al., 2007). No mammalian hosts have been determined in the Netherlands where infected ticks have been reported.

In the United States, an ehrlichial organism, closely related to ''*Candidatus* Neoehrlichia mikurensis'' based on a partial 16S rRNA sequence, was previously detected in raccoons (*Procyon lotor)* from Georgia (Dugan et al., 2005). Recently, this organism was isolated in ISE6 tick cell culture (Munderloh et al., 2007). In the current study, we investigated potential laboratory and wild hosts for the ehrlichial organism from raccoons. Based on the molecular and natural history uniqueness of this organism we propose that it is a novel species related to ''*Candidatus* Neoehrlichia mikurensis''.

2. Materials and methods

2.1. Experimental animals and procedures

Four, 10-week-old raccoons (RAC974, RAC975, and two negative controls) were acquired from a commercial source (Ruby Fur Farms, New Sharon, IA). Raccoons were kept in a climate-controlled, ectoparasite-free, animal housing facility at the College of Veterinary Medicine, University of Georgia (Athens, GA) for the duration of these studies. Raccoons were between the ages of 18 and 23 weeks of age when used in the experiments. Seven 16-week-old Wistar laboratory rats, seven 8-week-old BALB/c strain mice, ten 12-week-old C3H strain mice, and three young New Zealand white rabbits were obtained from a commercial vendor (Harlan, Indianapolis, IN). All experimental procedures were approved by the Institutional Animal Care and Use Committee. Animals were provided a species-appropriate diet of commercially available dry and canned food and water ad libitum.

2.2. Inoculum

The culture isolate (strain RAC413) used in this study was recently isolated in ISE6 tick cells from an experimentally infected raccoon (Munderloh et al., 2007). Uninfected and infected cultures were maintained at 34 °C in closed flasks (Greiner America, Longwood, NC), with L15B300 supplemented with 10% tryptose phosphate broth (Difco Laboratories, Detroit, MI), 5% heat-inactivated fetal bovine serum (Harlan, Indianapolis, Ind.), and 0.1% bovine lipoprotein concentrate (ICN, Irvine, CA), pH 7.2. Medium for infected cultures was additionally supplemented with 25 mM HEPES and 0.25% $NaHCO_3$ (Sigma, St. Louis, MO), and the pH was adjusted to 7.5–7.7 with 1N NaOH (Munderloh et al., 1999).

2.3. Experimental trials with raccoons

Prior to inoculation, the raccoons were shown to be negative for evidence of infection with or exposure to tick-borne disease agents by polymerase chain reaction (PCR) assay for the raccoon ehrlichial agent, *E. chaffeensis*, *E. canis, E. ewingii, Anaplasma*