Potomac Horse Fever: Identification and Transmission of the Causative Agent via Trematodes of Freshwater Snails

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Potomac horse fever was transmitted by inoculation of horses with trematodes from freshwater snails, and *Ehrlichia risticii* was recovered in cell culture. Water related sources of the agent of Potomac fever appear to be important in the transmission and life cycle of these agents. The agent(s) of Potomac horse fever consist of several strains of *Ehrlichia* species. Exiting vaccines contain only one strain from this group of agents. Author’s address: Dept. of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA 95616. © 1999 AAEP.

1. Introduction
The etiological agent of Potomac horse fever, also called equine monocytic ehrlichiosis, is a rickettsial agent currently termed *Ehrlichia risticii*. The disease was first reported along the Potomac River in Maryland in 1979 and has since been identified in other states and in Europe. Clinical signs include fever, anorexia, lethargy, mild colic with or without diarrhea, and sometimes laminitis. Abortion related to fetal infection has been described. The mode of transmission of this agent has remained unidentified. There is no evidence for spread of the disease by arthropod vectors such as ticks. Recent work has shown the close phylogenetic relationship of *Neorickettsia helminthoeca*, the agent of salmon poisoning in dogs, a frequently fatal enteric disease. We have recently described the detection of *E. risticii* DNA in tissues of freshwater operculate snails (Pleuroceridae: *Juga* spp.) and their trematode cercariae collected from stream waters on a PHF enzootic pasture in northern California and from the blood of a horse with clinical signs of PHF residing within that pasture. Here we describe the transmission of Potomac fever to horses via inoculation of trematode rediae and cercariae from freshwater snails and the development of fever, lethargy, anorexia, colic, and diarrhea. Sequence comparison of the 16S ribosomal RNA (rRNA) genes of the agent in the snails and that isolated from the blood of the experimentally infected horses was identical.

2. Materials and Methods
A. Snail Collection and Aquarium Culture
Freshwater snails were collected in August 1997 and October 1998 from a river coursing through a pasture in Weed, Siskiyou County, California, where previous cases of PHF had been confirmed. A total of 400 pleurocerid snails of the genus *Juga* were collected and transported in chilled stream water...
to our laboratory at the University of California, Davis. In the laboratory the snails were rinsed briefly and distributed into three freshwater aquaria. Water was prepared for snail growth and the snails fed algae pellets.

B. Source and Preparation of E. risticii Strains

In these experiments, snail secretions, tank water samples, and snail tissue were collected at different times and examined for the presence of E. risticii DNA by polymerase chain reaction (PCR). Blood and feces were collected from the horses throughout the experiment for PCR. Three E. risticii strains obtained from snails were used for comparison as previously described.4

C. Nested PCR Assays

A nested PCR that amplifies a 5’ segment (529 base pairs) of the 16S rRNA gene of E. risticii was used as an initial screen for the presence of E. risticii DNA in snail secretions and white blood cells of horses.12 Segments of two additional ehrlichia genes were amplified by PCR. Sets of nested primers were designed to detect portions of the E. risticii homologue of the Escherichia coli groESL heat-shock operon 35 and the E. risticii 51-kDa major antigen genes. Primer sequences and cloning and sequencing of amplified products are reported elsewhere.10 Sequences were subjected to BLAST analysis of GenBank nucleic acid sequences for similarity rank, percent identity, and deduced amino acid sequences (the later for the groESL and 51-kDa genes only).

D. Transmission

Three mature horses were attempted to be infected with trematode stages collected from PCR-positive snails. Each susceptible horse was inoculated by a different method (PO, SC, IP) and administered E. risticii PCR-positive snail secretions and monitored daily for clinical signs, hematology, clinical chemistry, PCR, cell culture detection of E. risticii, and serological status.

3. Results

E. risticii DNA was readily detectable by PCR in cercariae produced by the snails in room-temperature conditions. BLAST searches using the 16S rRNA gene sequences amplified from horse and snail samples consistently resulted in 98–100% homology with corresponding fragments of the 16S rRNA genes of E. risticii Illinois.

The subcutaneously inoculated horse became PCR positive for E. risticii infection, developed clinical signs of fever, anorexia, lethargy, decreased gut sounds, cow-like feces, ventral edema, and diarrhea and isolation of the E. risticii agent from blood in cell culture and in feces by PCR in both horses. The 16S rRNA gene sequence in the snail inoculated material was identical to the E. risticii strain isolated from the horse showing clinical signs of PHF. Continuous propagation of the agent in cell culture using a mouse murine macrophage cell line was successful.

4. Discussion

The identification of E. risticii DNA in snails and snail secretions suggested the potential importance of water borne infection. Our clinical cases of PHF had a history of water exposure or consumption from streams having the infected snails. The diversity of strains found is similar to the recent report of multiple strains of E. risticii isolated from horses with signs of PHF.10,11 The biological proof of the presence of the agents of Potomac horse fever in the trematode of Juga sp snails is confirmed by inoculation of snail rediae and cercariae into horses and development of clinical signs of Potomac horse fever, passage of the infection by blood to two other horses, and isolation of E. risticii in cell culture. Therefore the role of trematodes in the transmission of Potomac horse fever in other parts of the United States should be investigated.

Numerous strains of E. risticii have been identified.10,11 All vaccines for Potomac horse fever contain only one strain of E. risticii isolated from the blood of a single horse in 1984 in Illinois. Challenge studies for vaccine licensing have been performed by intravenous challenge. No testing for protection against oral transmission or with other pathogenic strains for horses has been performed. Vaccination has been considered marginally protective or nonprotective in the field.

References and Footnotes


