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IDENTIFICATION OF AN ENZOOTIC DIARRHEA ("Shasta River Crud") IN NORTHERN CALIFORNIA AS POTOMAC HORSE FEVER

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SUMMARY

A disease entity known as the “Shasta River crud” (SRC) has been recognized for at least 25 years in areas of Siskiyou and Shasta counties, in far northern California. It is characterized by anorexia, lethargy, diarrhea, variable fever, and in some cases laminitis, and is tetracycline responsive. The incidence is seasonal, with cases occurring in the spring and summer months near the Shasta and Klamath rivers. Limited serologic testing has provided some suggestion that the illness may be linked to *Ehrlichia risticii*, the agent of Potomac horse fever. Here we report the identification of *E. risticii* genomic DNA in blood buffy-coat cells from horses with SRC, and so provide the first definitive evidence that this enzootic diarrhea is in fact Potomac horse fever.

INTRODUCTION

For at least 25 years, veterinary practitioners in areas of Siskiyou and Shasta counties, California, have recognized a diarrheic disease of horses that is referred to locally as the “Shasta River crud” (SRC).1,2 It is seen in the spring

and summer months along the Shasta and Klamath rivers, with the early cases developing nearest the river and later cases occurring progressively farther away. The illness is characterized by signs of anorexia, lethargy, diarrhea, variable fever, and in some cases laminitis, and is responsive to systemic tetracyclines and intensive fluid therapy. Limited serologic testing (immunofluorescence assay, IFA) has provided some evidence that the disease may be linked to *Ehrlichia risticii*, the agent of Potomac horse fever.1,3 Owing to the remarkably high false-positive rate of the *E. risticii* IFA,4 however, as well as an absence of confirmatory findings (e.g., isolation or detection of the causative agent), the etiology of this seasonally-recurring illness has remained unclear.

Here we report the identification of *E. risticii* DNA in blood buffy-coat cells from two horses with clinical signs of SRC. The agent was detected by a nested polymerase chain reaction (PCR),2 and by Southern hybridization using an internal oligonucleotide probe2; final confirmation of identity was provided by DNA sequencing. Sequencing results further indicated that the SRC agent is most closely related to the Kentucky strain of *E. risticii*.

MATERIALS AND METHODS

Case reports

Case No. 1 (“Shorty”). In early May 1996, a 10-
year-old Quarter Horse gelding living along the Shasta River in Weed, California (Siskiyou County), was examined for an illness of 1-2 days' duration. The horse had resided in the county for only a year and had been vaccinated against *E. risticii* by its owner. Clinical findings included lethargy, anorexia, and diarrhea. Four other horses on the ranch had died with similar signs, several years previously. The present horse had been vaccinated against *E. risticii* by its owner. Clinical findings included a rectal temperature of 101.8°F, a pulse rate of 60, and a respiratory rate of 30. Moderate scleral injection was noted. Decreased abdominal sounds and a profuse, watery diarrhea were evident. Digital pulses were normal. A fecal sample was positive for strongyles and protozoa. The horse was treated with intravenous fluids, a non-steroidal antiinflammatory agent, and oxytetracycline. A blood sample for PCR was obtained prior to oxytetracycline therapy. The horse responded to therapy and made an uneventful recovery.

**RESULTS AND DISCUSSION**

Blood Buffy-coat cells from both cases of SRC produced amplified 529-bp bands characteristic of *E. risticii* (Figure 1). Southern hybridization and DNA sequencing revealed that the 529-bp bands were indeed 16S ribosomal RNA (rRNA) gene of *E. risticii*. Appropriate positive and negative controls were included in each PCR run. Preliminary verification of identity of the amplified products was provided by Southern hybridization using an internal oligonucleotide probe. For final verification of identity the majority of the 16S rRNA gene from case no. 2 was sequenced, as previously described.

**Table 1. Nucleotide differences within the 16S rRNA genes of the "Shasta River crud" agent and strains of *Ehrlichia risticii***

| Nucleotide at position: | 76 | 77 | 90 | 92 | 97 | 131 | 619 | 956 | 971 | 1221 | 1231 | 1246 |
|-------------------------|----|----|----|----|----|-----|-----|-----|-----|------|------|------|
| **E. risticii strain**  |    |    |    |    |    |     |     |     |     |      |      |      |
| Illinois                | G  | G  | C  | T  | C  | G   | G   | T   | G   | C    | C    | G    |
| SRC agent               |    |    |    |    |    |     |     |     |     |      |      |      |
| Kentucky                |    |    |    |    | A  | A   | C   | T   | T   | A    |      |      |
| Ohio-081                | A  | A  | T  | C  |    |     |     |     |     | A    | T    | A    |

Periods indicate conserved positions relative to *E. risticii*-Illinois, the type strain.
ences were found between the SRC agent and the E. risticii type strain (Illinois), while 6 were noted with the more distantly related Ohio 081 strain (Table 1).

Anorexia, lethargy, diarrhea, and fever are major clinical findings in Potomac horse fever\textsuperscript{2,3,7,10} and represent the major clinical signs of SRC as well. Similarly, laminitis is a significant complication in a percentage of cases.\textsuperscript{8,10} Interestingly, the gradual spread of SRC farther away from the river as the season progresses is an exact echo of the initial descriptions of Potomac horse fever along the Potomac River.\textsuperscript{8}

Horses with SRC are indistinguishable clinically from Potomac horse fever cases we have identified in nearby Klamath Falls in southern Oregon,\textsuperscript{6} where the disease is known locally as “ditch fever” from its association with pastures bordering irrigation ditches. The Klamath Falls area is contained within a northern excursion of the Siskiyou/Shasta ecosystem, a combined upper Sonoran and transition zone that sweeps across portions of southern Oregon but is inhabited by vegetation and wildlife that are clearly Californian.\textsuperscript{11} Although the vector of E. risticii remains unidentified,\textsuperscript{10} it seems reasonable to suspect that if it is present in Klamath Falls, it may be present in Siskiyou and Shasta counties as well. Available data suggest that the vector is not a tick, but is instead an organism closely associated in some way with river and irrigation water.\textsuperscript{2,10}

The nucleotide sequences of 16S rRNA genes are known to vary in an orderly manner throughout the phylogenetic tree; as “molecular chronometers” they exhibit only minor sequence divergence among strains of an individual species.\textsuperscript{12} Three major groups of E. risticii based on 16S rRNA gene sequence differences have been described.\textsuperscript{5} These include the type strain (Illinois) and related Ohio isolates; the Kentucky strain; and a more variable strain known as Ohio 081. The genetic differences among these strains are mirrored in their antigenic differences.\textsuperscript{5,12} It has been suggested that the degree of divergence shown by the Kentucky and Ohio 081 strains from E. risticii-Illinois may justify their removal from the species risticii and reassignment to separate species status.\textsuperscript{5} Thus it is possible that Potomac horse fever (and thus SRC) may be caused by more than a single species of the genus Ehrlichia. Isolation and cultivation of various strains of E. risticii from different areas of the country should improve our understanding of the genetic and antigenic divergence of the organism, and aid in the development of more effective vaccines and serodiagnostic tests.

To summarize, we have provided the first DNA-based evidence that the disease entity known to practitioners in far northern California as the “Shasta River crud” is in fact Potomac horse fever. This part of California may be one of the few areas of the state where conditions necessary for the propagation and transmission of E. risticii and its reservoir hosts/vectors exist; overall the occurrence of Potomac horse fever in California horses appears to be low.\textsuperscript{4} Considering that SRC has been recognized in the region for at least 25 years, its clinical discovery appears to predate that of Potomac horse fever itself, which was first reported only in 1979.\textsuperscript{8} It is thus interesting to reflect how, but for the vicissitudes of history, the disease caused by E. risticii might just as easily have been named “Shasta horse fever.”

Addendum. Since this work was performed we have identified E. risticii by PCR in three additional cases of SRC in Siskiyou County. The first was a febrile 21-year-old Quarter Horse gelding pastured at Horse Creek along the Klamath River, northwest of Mt. Shasta (sample provided by Dr. Paul Miller). The second was a 4-year-old Quarter Horse mare from Montague, along the Shasta River (sample provided by Dr. Bob Cook, Yreka Veterinary Hospital, Yreka, CA). The third was a febrile 15-year-old Quarter Horse gelding that has lived along the Shasta River for many years (sample provided by Dr. Miller).

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