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Equine Granulocytic Ehrlichiosis in Connecticut Caused by an Agent Resembling the Human Granulocytotropic Ehrlichia

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The first recognized cases of equine granulocytic ehrlichiosis in New England are described. The DNA sequence of the 16S rRNA gene of the causative ehrlichia was found to be identical to that of the human granulocytotropic ehrlichia, the agent of human granulocytic ehrlichiosis.

Equine granulocytic ehrlichiosis (EGE) is a seasonal, largely self-limiting disease of horses that is caused by a granulocytotropic rickettsia, *Ehrlichia equi* (7, 9, 16). In California, EGE occurs almost exclusively in certain well-defined geographical areas (enzootic foci or "hot zones") during the late fall, winter, and spring and is probably transmitted by ticks (10, 14). The clinical picture typically includes fever, reluctance to move, lethargy, ataxia, distal limb edema, thrombocytopenia, and petechiation. Diagnosis is based on the clinical signs, seasonality, geographic location, and identification of inclusion bodies (morulae) in the cytoplasm of circulating neutrophils (9). An indirect immunofluorescent antibody test of paired serum samples can be used retrospectively to confirm recent infection (3, 11). Treatment with tetracycline antibiotics induces a prompt defervescence, with a more gradual resolution of other clinical signs. The mortality rate is low unless secondary infection or a severe injury precipitated by incoordination should occur.

The first cases of EGE were observed in northern California in the 1960s (7, 15, 16). Since that time, the disease has been diagnosed in a few other states as well as in Europe and South America (9). Here we describe what are to our knowledge the first diagnosed cases of EGE in New England. These cases represent the vanguard of a localized outbreak that eventually numbered over 50 horses. The 16S rRNA gene of the causative ehrlichia was sequenced and shown to be identical to that of the human granulocytotropic ehrlichia, the agent of human granulocytotropic ehrlichiosis (HGE) (2, 5, 13, 17).

Index case. In late November 1994, a 30-year-old Quarter-horse-cross gelding (case 1) in Connecticut was found to be febrile (105°F [41°C]), anorectic, and icteric, with an elevated heart rate of 48 beats per min. The horse had a history of tick exposure and was stabled in a closed environment with two other horses, neither of which was symptomatic. Hematologic examination revealed a leukocyte count of 5,600/μl with lymphopenia, thrombocytopenia (75,000 platelets per μl), and an elevated plasma fibrinogen level (500 mg/dl). Rare cytoplasmic inclusion bodies characteristic of *E. equi* (10) were observed in circulating neutrophils. Blood buffy coat cells were strongly positive for *E. equi* or the HGE agent by the nested PCR (Fig. 1). The nested PCR amplifies a 928-bp sequence of the 16S rRNA gene of both *E. equi* and the HGE agent (3, 12). Cloning and sequencing of the PCR product indicated that it was more closely allied to the HGE agent (2, 5, 17, 18) and the granulocytotropic ehrlichia of horses and dogs in Sweden (8) than to California strains of *E. equi* (1). This distinction is based in particular on two critical regions at the 5′ and 3′ ends of the 16S rRNA gene (Table 1) (5). The gelding was treated with tetracycline. The temperature returned to normal within 24 h, and the horse made an uneventful recovery.

Subsequent cases. Once the first case was observed, four other adult Connecticut horses with EGE were rapidly brought to our attention. Case 2 presented with fever, anorexia, an elevated heart rate, and a history of tick exposure. The horse was housed in a stable of approximately 20 horses, one of which had been ill the previous week with fever and limb edema. The PCR with blood buffy coat cells was positive (Fig. 1). Case 3 had a high fever, elevated heart rate, digital pulse in all four feet, icterus, and evidence of tick attachment. Residual cells in collected serum were separated by centrifugation and processed for PCR, which gave a strongly positive result (Fig. 1). Case 4 presented with fever, anorexia, elevated heart rate, edema of all four limbs, and evidence of tick bites. The PCR with residual cells from serum was positive (Fig. 1). Case 5 had a low-grade fever, elevated heart rate, edema of all four limbs, and thrombocytopenia, leukopenia, and anemia. A fivefold rise in the indirect immunofluorescent antibody titer to *E. equi* (from 1:20 to 1:640) over a 3-week period was demonstrated. Clinical signs in affected horses usually resolved quickly after treatment with tetracycline or oxytetracycline.

Discussion. The HGE agent, which is most likely transmitted by ticks (13, 18), has been identified as a cause of morbidity and mortality in the upper midwestern and northeastern United States (2, 5, 6, 17, 18). Recently, we have shown that the HGE agent when inoculated into horses produces a disease indistinguishable from that caused by *E. equi*, confirming this ehrlichia of human origin as a cause of EGE under experimental conditions (4, 12). The present report suggests further that the agent may be responsible for field cases of EGE as well, particularly in areas of the country where HGE is prevalent. Available evidence indicates that *E. equi* and the HGE agent are very closely related and probably represent strains of the same organism (1, 5, 6, 12). Comparisons of the 16S rRNA gene sequences of the two ehrlichiae have revealed only three nucleotide differences between them (5), while serologic studies have demonstrated that the HGE agent and *E. equi* share significant antigenicity (2, 6). The biological relationship between the two strains has been strengthened by evidence in the

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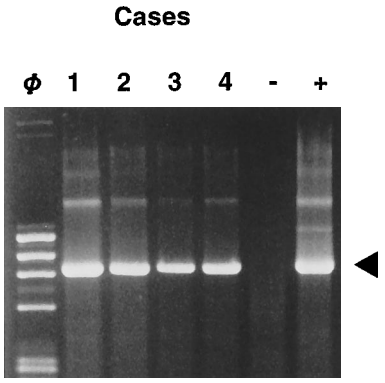

FIG. 1. PCR of *E. equi* or HGE agent with DNA from blood buffy coat cells (cases 1 and 2) or residual cells in serum (cases 3 and 4). The arrowhead indicates the position of the 928-bp nested PCR product. −, negative control; +, positive control; ϕ, ϕX174 replicative form DNA-*Hae*III digest.

horse that prior infection with the HGE agent protects against challenge with *E. equi* (4).

Members of the equine species are thought to represent dead-end hosts for *E. equi*, with the organism being maintained in some as yet unidentified wildlife reservoir, e.g., deer or rodents. Because of the presence of ticks on a large percentage of infected horses and the seasonality of clinical illness, tick transmission has been suspected but has remained largely unproven (10). Recently, however, we have shown experimentally that the western black-legged tick *Ixodes pacificus* can transmit *E. equi* between horses, with EGE developing in the recipient (14). We have identified *E. equi* by PCR in several adult, laboratory-reared *I. pacificus* ticks that as nymphs had been fed on an experimentally infected horse (3, 14). Field studies are currently in progress to test the hypothesis that *I. pacificus* is an important vector of *E. equi* in areas of northern California where EGE is enzootic. The ticks found on the horses in the present report were not identified; however, members of the genus *Ixodes* (e.g., *Ixodes scapularis*) are commonly active in the fall in New England and thus represent potential vectors of granulocytotropic ehrlichiae in the northeastern United States.

We conclude that the minor genetic differences in the 16S rRNA genes of *E. equi* and the HGE agent may reflect the genetic variability inherent in a single granulocytotropic ehrlichia that is widespread throughout the country. Furthermore, we hypothesize that cases of human illness now attributed to human granulocytotropic ehrlichia may in actuality represent tick-borne infections with a geographically diverse ehrlichial species known chiefly as an equine pathogen.

TABLE 1. Nucleotide sequence comparison of critical 5′- and 3′-end sequences of the HGE agent, Swedish ehrlichia, and *E. equi* 16S rRNA genes with the homologous amplified PCR product from the Connecticut EGE agent

DNA source	DNA sequence[a]	
	5′ end	3′ end
HGE agent[b]	79-GAATAGTTAGT-89	881-CAAGCGGTGGA-891
Swedish ehrlichia[c]	79-. . . . . -89	881-. . . . . -891
Connecticut EGE agent	79-. . . . . -89	881-. . . . . -891
E. equi[d]	79-. . . . . A. . . . . -89	881-. . . . . - . . . . . -891[e]

[a] Numbers represent the flanking nucleotides with reference to positions in the 16S rRNA gene of the HGE agent (5) (GenBank accession no. U02521). Conserved positions are indicated by periods.
[b] Ehrlichial agent of HGE (5, 12).
[c] As described in reference 8.
[d] California strain of *E. equi* (1) (GenBank accession no. M73223).
[e] No nucleotide corresponds to HGE position 886 in *E. equi* (5).

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