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Protection against *Ehrlichia equi* Is Conferred by Prior Infection with the Human Granulocytotropic Ehrlichia (HGE Agent)

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A Thoroughbred filly that developed clinical signs of equine granulocytic ehrlichiosis following inoculation with the human granulocytotropic ehrlichia was shown to be resistant to challenge with *Ehrlichia equi*, a closely related agent. This result further substantiates the close and potentially conspecific relationship between these two granulocytotropic ehrlichiae.

Equine granulocytic ehrlichiosis (EGE) is a seasonal, normally self-limiting disease of horses that was first reported in northern California in the 1960s (6, 8, 9, 11, 16). Clinical signs include fever, depression, icterus, reluctance to move, ataxia, distal limb edema, thrombocytopenia, and petechiation. In California, EGE is found almost exclusively in certain well-defined geographical areas ("hot zones") during the late fall, winter, and spring. The cause of the disease is a granulocytotropic rickettsia, *Ehrlichia equi*, which is thought to be transmitted by hard-bodied (ixodid) ticks (11, 15). Horses appear to act as dead-end hosts, with the organism being maintained in some as yet unidentified wildlife reservoir, e.g., deer or rodents. Immunity to *E. equi* is known to persist for at least 2 years and is not associated with a latent infection or chronic carrier state (6, 14). Limited studies suggest that passive immunity is transferred to foals in colostrum but is of relatively short duration (6). Virtually nothing is known about strain variation in *E. equi*, with all studies of immunity to date describing challenge only with the homologous strain.

Human GE (HGE) is an emerging human disease that is caused by an ehrlichia very similar to *E. equi* and is probably transmitted by ticks (2, 4). Comparison of the 16S rRNA gene sequences of the HGE agent and *E. equi* have revealed only three nucleotide differences between them (4), while serologic studies have demonstrated that the two organisms share significant antigenicity (2, 5). Recently we have shown that the HGE agent causes a disease in horses that is indistinguishable in evolution and character from EGE caused by *E. equi* (13). Taken together, these data suggest that *E. equi* and the HGE agent are very closely related and may represent a single species (4, 7). To investigate this relationship further, we performed an experiment to determine whether a horse that has recovered from HGE infection is immune to challenge with *E. equi*.

A 1-year-old Thoroughbred filly seronegative for *E. equi*-HGE agent antibodies (12) was inoculated intravenously with 2 ml of thawed equine neutrophils containing the HGE agent (BDS strain). This inoculum was derived from a horse that had been inoculated with fresh whole blood from a human patient with HGE (13). The filly was monitored twice daily by animal caretakers for clinical signs of illness. Blood samples were drawn each morning for routine hematologic, serologic, and PCR assays. Hematologic parameters included erythrocyte, leukocyte, and platelet counts, icterus index, and microscopic examination for the presence of ehrlichial inclusion bodies (morulae) in the cytoplasm of neutrophils (11). *E. equi*-HGE agent antibodies were detected by an indirect immunofluorescent-antibody assay, essentially as described elsewhere (3, 12). A nested PCR for *E. equi*-HGE agent genomic DNA in blood buffy-coat cells was performed as described elsewhere (3).

The filly remained clinically asymptomatic until a fever (104.5°F [40.3°C]) developed on day 10 postinoculation. Depression, reluctance to move, mild icterus, and the presence of morulae in neutrophils were noted shortly thereafter. The morulae reached a peak on day 14 postinoculation, with 75% of neutrophils containing visible inclusions. Platelet counts declined to 25,000/μl (normal range, 100,000 to 300,000/μl) by day 15. The nested PCR with blood buffy-coat cells became positive on day 9 (1 day before the onset of fever and 2 days before morulae were first observed) and remained positive through day 18 (Fig. 1A), by which time the clinical signs were resolving. The serum antibody titer rose to 1:80 by day 26, eventually reaching a plateau at 1:160. The overall clinical picture was identical to that of horses experimentally inoculated with *E. equi* (3, 6, 16). The onset of both clinical signs and PCR positivity was slightly delayed in comparison with the onset in our previous study of the HGE agent (13), probably because a lower dose of inoculum was administered.

Eight weeks after recovery, the filly was challenged with *E. equi*. Dextran-separated neutrophils (6 ml) from a horse that had been experimentally infected with a California strain of *E. equi* designated MRK (12) were retrieved from liquid-nitrogen storage and divided into two inocula of 3 ml each. One inoculum was administered intravenously to the filly, and the second was administered intravenously to a 5-year-old, *E. equi*-seronegative Appaloosa mare used as an inoculum control. The filly remained clinically asymptomatic, hematologically normal, and PCR negative (Fig. 1B) for the duration of the challenge study and for a 3-month observation period thereafter. Her serum antibody titer, however, rose from 1:160 to ≥1:640, consistent with an anamnestic response to ehrlichial antigens. By contrast, the Appaloosa mare developed clinical

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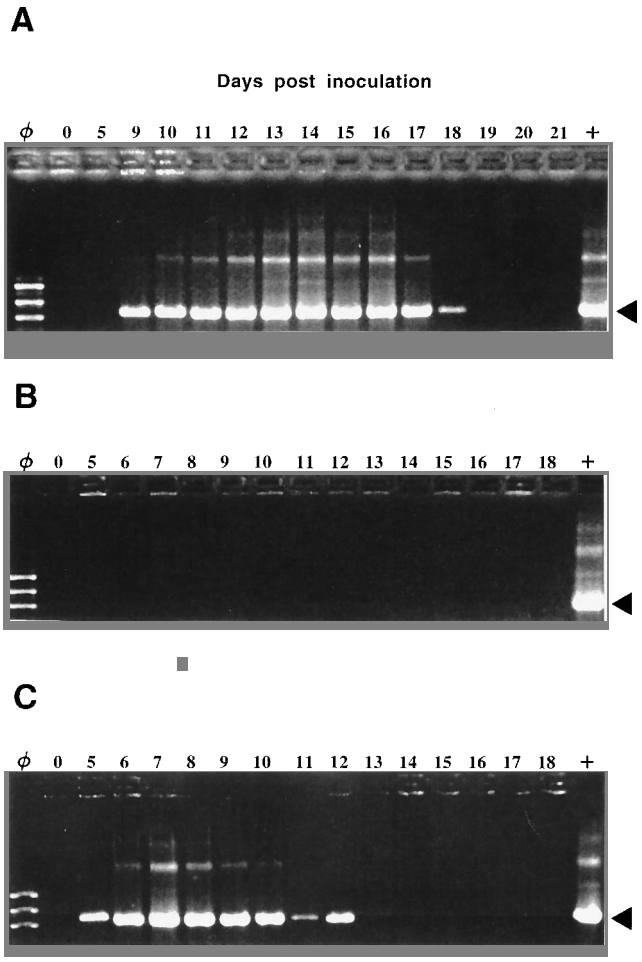

FIG. 1. *E. equi*-HGE agent nested PCR performed with serial blood buffy-coat samples from experimentally infected horses. Results with samples from the Thoroughbred filly after inoculation with the BDS strain of the HGE agent (A), the filly after subsequent challenge with *E. equi* MRK (B), and the Appaloosa mare after inoculation with *E. equi* MRK (C) are shown. Arrowheads indicate the position of the ehrlichial amplicon (928 bp). Lanes +, positive *E. equi* control; lanes ϕ, *Hae*III-digested ϕX174 replicative-form DNA.

signs, thrombocytopenia, and ehrlichemia (Fig. 1C) characteristic of experimentally induced EGE.

These data indicate that prior infection with the HGE agent cross-protects against subsequent challenge with *E. equi*, a finding that further substantiates the close and potentially conspecific relationship between these granulocytotropic ehrlichiae. Such results reflect the virtual identity of these two agents as revealed by serologic and genetic methods; i.e., the HGE agent and *E. equi* share significant antigenicity, as shown by indirect immunofluorescent-antibody and immunoblot assays, and have nearly identical 16S rRNA gene sequences (4, 5). In a recent study, it was found that an ehrlichia causing granulocytic ehrlichiosis in horses and dogs in Sweden is identical to the HGE agent in its 16S rRNA gene sequence (7). We have obtained a similar result for an ehrlichia causing EGE in the northeastern United States (10). The California (MRK) strain of *E. equi* that has been used in most reported inoculation and sequencing studies (1, 4, 6, 14, 16) thus appears to differ marginally in its 16S rRNA gene sequence from HGE agent-like strains found in the midwestern and eastern United States and in Europe, although it produces an identical disease in horses. The HGE agent is capable also of causing morbidity and mortality in humans (2, 4), and we hypothesize that *E. equi* may share this feature. On the basis of these findings, we speculate that the HGE agent and *E. equi* represent variants of a geographically diverse ehrlichial species that is widespread throughout the Americas and Europe and is able to infect humans as well as animals.

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