Human Granulocytic Ehrlichiosis Agent Infection in a Pony Vaccinated with a *Borrelia burgdorferi* Recombinant Ospa Vaccine and Challenged by Exposure to Naturally Infected Ticks

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A pony was vaccinated with recombinant Ospa vaccine (rOspa) and then exposed 3 months later to *Borrelia burgdorferi*-infected ticks (*Ixodes scapularis*) collected in Westchester County, N.Y. At 2 weeks after tick exposure, the pony developed a high fever (105°F). Buffy coat smears showed that 20% of neutrophils contained ehrlichial inclusion bodies (morulae). Flunixin Meglumine (1 g daily) was given for 2 days, and the body temperature returned to normal. PCR for ehrlichial DNA was performed on blood samples for 10 consecutive days beginning when the pony was first febrile. This pony was monitored for another 3.5 months but developed no further clinical signs. The 44-kDa immunodominant human granulocytic ehrlichiosis antigen gene was amplified by PCR and cloned into a pCR2.1 vector. DNA sequence analysis of this gene showed it was only 8 bp different (99% identity) from the results reported by others (J.W. Ijdo et al., Infect. Immun. 66:3264–3269, 1998). Western blot analysis, growth inhibition assays, and repeated attempts to isolate *B. burgdorferi* all demonstrated the pony was protected against *B. burgdorferi* infection. These results highlight the potential for ticks to harbor and transmit several pathogens simultaneously, which further complicates the diagnosis and vaccination of these emerging tick-borne diseases.
mogen tetramethylbenzidine with $H_2O_2$ as a substrate was measured kinetically and expressed as the slope of the reaction rate between enzyme and substrate solution. Each unit of slope was designated as a KELA unit.

The procedure for the Western blot analysis was done as previously described (4, 5, 7). French-pressed *B. burgdorferi* lysate was used as an antigen and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (4, 5, 7). Western blot analysis was performed in a miniblottter (4, 5, 7). Test sera from experimental animals were used as a first antibody, followed by goat anti-horse immunoglobulin G conjugated to horseradish peroxidase as a second antibody.

**PCR and sequence of 44-kDa protein gene for HGE agent.** Venous blood was collected into ACD tubes. DNA template derived from the blood samples was prepared by using DNAzol according to the manufacturer's instructions (Life Technologies, Rockville, Md.). PCR with the HGE agent-specific Ger3 and Ger4 primer set for 16 S RNA gene was performed on blood obtained when the pony was febrile, as previously described (14). Based on published data, two additional primers (sense, 5'-ATGTCTATGGCTATAGTCATGGCT-3'; antisense, 5'-CT TAAAGCAAGCCTAACACC-3') were designed to amplify the 44-kDa immunodominant protein gene (17). The amplified PCR product was ligated into a pCR2.1 vector (Invitrogen), and both strands of the cloned DNA were completely sequenced by using the Applied Biosystems model 373A automated DNA sequencing system. The thermal cycling of the sequencing reactions utilized the Tag DyeDeoxy terminator cycle sequencing kit.

**Pathology and histopathology.** As part of the overall rOspA vaccine study, this pony was euthanized 3.5 months after tick exposure and then necropsied. The following tissues were fixed in 10% neutral buffered formalin: joint capsules (right and left elbow, shoulder, stifle, carpus, tarsus, and fetlock), cerebellum, cerebrum, meninges, spinal cord, myocardium, urinary bladder, thyroid, liver, spleen, kidney, lung, stomach, intestine, skeletal muscles, aorta, eyes, nerves (left and right brachial plexus, trigeminal ganglion, cervical and thoracic nerve roots, median, ulnar, radial, Tibial, fibular, sciatic, and facial), and lymph nodes (axillary, prescapular, and popliteal). Tissues were embedded in paraffin wax, sectioned, and stained with hematoxylin and cosin by conventional methods for histopathologic evaluation.

**RESULTS**

**Clinical signs and examination of buffy coats.** Two weeks after tick exposure, this pony had a high fever (105°F). Examination of Giemsa-stained buffy coats on the first day of fever revealed that 20% of the neutrophils had visible morulae, a finding compatible with HGE agent infection (Fig. 1). For the next 3 days, morulae remained visible in about 10% of the neutrophils, but Giemsa-stained buffy coats were negative when examined 10 days after the initial fever.

**Serology.** Western blots confirmed that the pony developed an appropriate antibody response to rOspA vaccination, while unvaccinated controls developed typical Western blot patterns seen only with infection. Western blot analysis with *B. burgdorferi* B31 whole-cell lysate showed that OspA antibody appeared 3 weeks after the first vaccination (Fig. 2, lane 2). Bands became denser after the second vaccination (Fig. 2, lane 5). Bands also appeared in the 20- and 60-kDa regions (Fig. 2). Western blot analysis with HGE agent whole-cell lysate (NCH-1 strain) showed antibody reactivity at approximately 29, 37, 38, and 44 kDa and faintly at 110 kDa (Fig. 3, lanes 12 and 13), but no bands formed when DH82 cell lysate was used as a control (data not shown).

**PCR and DNA sequence.** PCR was positive for ehrlichial DNA when the pony was first febrile at 14 days after tick exposure (Fig. 4). Ten days later, we could no longer detect the PCR product (Fig. 4, lane 24). The DNA sequence indicated only 8 bp differences from previously reported DNA sequence.

**Histopathology.** No significant gross or histopathologic lesions were seen in this pony 3.5 months after infection.

**DISCUSSION**

Here we report HGE agent infection in a horse vaccinated with recombinant OspA vaccine and then exposed to field-collected adult *I. scapularis* ticks harboring both *B. burgdorferi* and the HGE agent. This case highlights the continued importance of vaccinating individuals against Lyme disease to avoid tick exposure, since ticks harbor multiple potential pathogens. Failure to do so may result in infection with pathogens other than the Lyme agent. Reportedly, horses that recovered from HGE agent infection are protected against reinfection (2). However, Horowitz et al. recently reported that a woman was reinfected with HGE agent 2 years after the first infection (16). Revaccination may be necessary to fully protect animals or
humans. HGE agent infection may cause immunosuppression (36). However, Holmeister et al. reported that mice coinfected with B. burgdorferi and HGE agent only had greater spirochete distribution without increasing the severity of Lyme disease (E. K. Holmeister, M. H. Moro, D. Mathiesen, S. W. Barthold, and D. H. Persing, Abstr. 7th Int. Cong. Lyme Borreliosis, abstr. F1008, 1996). Whether this is the case in humans, dogs, and horses is currently unknown.

Western blot analysis with whole B. burgdorferi cell lysates also showed bands in the 20- and 60-kDa regions, which were probably degraded products and dimers of OspA as previously described (4). Western blot analysis with HGE agent whole-cell lysate (NCH-1) indicated that there were strong bands at 10 weeks after tick challenge (17, 18, 23), but no bands were present when uninfected DH82 cell lysates were used as antigens (data not shown). Thus, the bands seen in the Western blot with HGE agent-infected cell lysates as antigens were specific for the HGE agent. The 44-kDa immunodominant protein is heterogeneous among different HGE agent isolates (1, 38). It has been reported that a minimum copy number of this 44-kDa protein gene is 18 and that five different mRNAs are transcribed from 44-kDa protein genes in HL-60 cell cultures (37). Two different 44-kDa homologous proteins are expressed in the HL-60 cell cultures, which are regulated at the transcriptional level (37). It is currently unknown if antibodies against the 44-kDa immunodominant protein are protective. Further study is required to ascertain if this antigen could be used in combination with OspA as a recombinant vaccine to protect people and animals against both diseases.

No histopathologic lesions were found at the time of necropsy in this pony. Lesions associated with HGE agent infection would most likely be found when the patient is febrile (15). However, we monitored the pony for 3.5 months after B. burgdorferi-infected tick exposure in order to determine if this pony was protected from Lyme disease after vaccination with an OspA vaccine.

In conclusion, EGE occurred in a pony vaccinated with a recombinant OspA vaccine and challenged by exposure to
field-collected adult ticks. Caution still needs to be taken to prevent tick bites in order to avoid transmission of other tickborne diseases.

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