

Brief Report

PERINATAL TRANSMISSION OF THE AGENT OF HUMAN GRANULOCYTIC EHRLICHIOSIS

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HUMAN granulocytic ehrlichiosis was first described in the United States, in the northern Midwest, in 1994.¹ Human granulocytic ehrlichiosis is caused by an organism, still referred to as the agent of human granulocytic ehrlichiosis, that is similar to two animal pathogens, *Ehrlichia phagocytophila* and *E. equi*.²⁻⁴ Transmission of human granulocytic ehrlichiosis occurs through the bites of ixodes ticks, which are the arthropod vectors for *Borrelia burgdorferi* and *Babesia microti*.^{5,6} Human granulocytic ehrlichiosis is an acute, febrile, nonspecific illness that may be severe enough to cause hospitalization and even death, particularly in the elderly.^{1,7,8} We describe a case of human granulocytic ehrlichiosis that developed in a pregnant woman near term and was transmitted perinatally to her infant.

CASE REPORTS

Mother

A 35-year-old woman, 39 weeks pregnant, was admitted to the hospital on October 4, 1997, at the onset of uterine contractions. She had malaise and had been feverish earlier that day. She had had an episode of Lyme disease with erythema migrans and antibodies to *B. burgdorferi* 10 years earlier and had a history of urinary tract infections. She lived in a tick-infested area of Connecticut. She recalled finding ticks crawling on her one week before admission, but none had embedded themselves in her skin. On admission her temperature was 38.1°C. Laboratory studies were limited to a complete blood count, which revealed a white-cell count of 6300 per cubic millimeter (normal range, 4600 to

11,200), with 5 percent lymphocytes, 75 percent neutrophils, and 17 percent band forms; a hematocrit of 35 percent (normal range, 36.4 to 45.8); and a platelet count of 168,000 per cubic millimeter (normal range, 160,000 to 410,000). Urinalysis revealed no white cells. On the following day, the patient had a normal vaginal delivery without complications. Fetal-scalp monitoring was not used. Therapy with clindamycin and gentamicin was begun because of persistent fever. The physical examination was normal, and there were no rashes. A chest roentgenogram, sinus radiographs, and pelvic magnetic resonance images were interpreted as normal. Two sets of routine blood cultures obtained that day were negative. A cervical culture was positive for group B streptococcus.

On the day after delivery, the woman's temperature rose to 40.6°C and she reported chills, malaise, fever, and myalgias. The results of a physical examination again were unremarkable. On that day, her white-cell count was 5600 per cubic millimeter, her hematocrit was 32 percent, and her platelet count was 130,000 per cubic millimeter. Over the next four days, she remained febrile without an identified source of infection. However, she noted some gradual improvement in her systemic symptoms. On October 9, she had a white-cell count of 5000 per cubic millimeter with 20 percent lymphocytes and a platelet count of 98,000 per cubic millimeter. On October 10, her alkaline phosphatase level was 186 U per liter (normal range, 30 to 115), her aspartate aminotransferase level was 74 U per liter (normal range, 7 to 40), her alanine aminotransferase level was 68 U per liter (normal range, 7 to 40), and her lactate dehydrogenase level was 420 U per liter (normal range, 100 to 225). On October 9, a whole-blood sample obtained on October 7 and treated with acid-citrate-dextrose as an anticoagulant demonstrated DNA from the agent of human granulocytic ehrlichiosis on polymerase-chain-reaction (PCR) assay, and a buffy-coat preparation from October 9 revealed morulae in granulocytes. The following day, clindamycin and gentamicin therapy was discontinued, and doxycycline treatment (100 mg orally twice daily) was started. Within 24 hours the patient became afebrile. She was treated for five days and remained well eight months later. The agent of human granulocytic ehrlichiosis was identified in a culture three days after inoculation with a sample taken on October 10, before the beginning of doxycycline therapy.

Sequential serologic indirect-immunofluorescence assays for the agent of human granulocytic ehrlichiosis demonstrated an increase in the antibody titer from 1:80 on October 10 to 1:2560 or more on October 17. An enzyme-linked immunosorbent assay (ELISA) did not detect IgG or IgM antibodies to *B. burgdorferi* in serum obtained on October 10. However, an ELISA of serum obtained on October 17 was positive for both IgG and IgM antibodies, and Western blotting was positive for IgM, with bands of 93, 66, 41, 39, 35, 29, and 24 kd (that at 24 kd indicates the presence of outer surface protein C [OspC]). *B. burgdorferi* was not detected by PCR in whole blood obtained on October 10.

Infant

On October 5, a 3000-g girl was born to the patient. The Apgar scores were 8 and 9 at one and five minutes, respectively. Breast-feeding was stopped after 24 hours because of the mother's illness and was not resumed because of the mother's use of doxycycline. The expressed breast milk was discarded. At six days of life, the infant was discharged with her mother. The next day the mother noted that the baby felt warm but fed well. On October 13, the child's ninth day of life, she had a temperature of 39.4°C and was referred for admission.

The physical examination on admission was normal. Treatment with ampicillin and gentamicin was begun after a workup for sepsis. A complete blood count revealed a white-cell count of 5200 per cubic millimeter, with 37 percent neutrophils, 12 percent band forms, and 39 percent lymphocytes, 10 percent monocytes, and 1 percent metamyelocytes; a hematocrit of 37.2 percent; and a platelet count of 92,000 per cubic millimeter. Liver enzyme lev-

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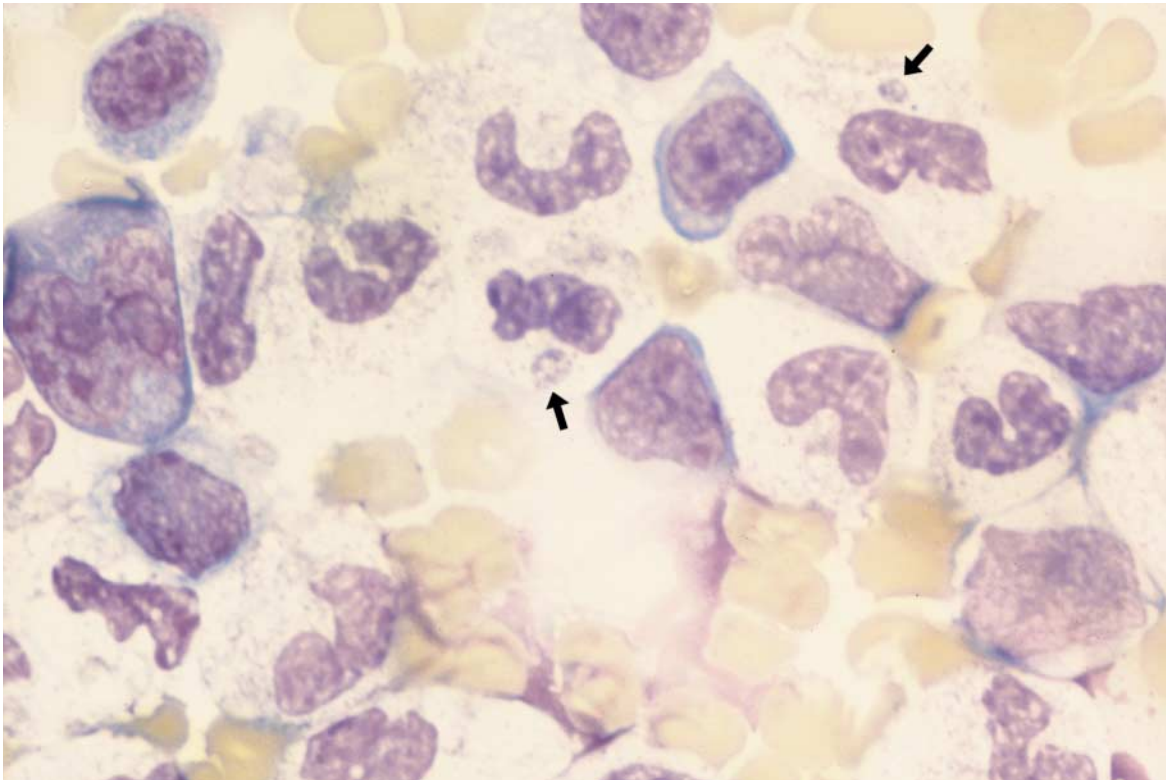


Figure 1. Photomicrograph of a Buffy-Coat Smear Stained with Wright's Stain, Showing the Infant's Granulocytes Infected with the Agent of Human Granulocytic Ehrlichiosis ($\times 1000$). Arrows indicate morulae.

els, urine, and cerebrospinal fluid were normal. The cerebrospinal fluid was not examined for morulae. Because human granulocytic ehrlichiosis had been diagnosed in the mother, a buffy-coat smear of the infant's blood was examined, revealing morulae in 23 percent of granulocytes (Fig. 1). A retrospective PCR analysis of the infant's blood spot obtained at birth did not detect DNA of the agent of human granulocytic ehrlichiosis.

After the benefits and risks had been considered, intravenous doxycycline treatment (5 mg per kilogram of body weight per day, divided into two doses) was begun. Within 24 hours, the baby's body temperature returned to normal and her condition was clinically improved. The platelet count and neutrophil count reached nadirs of 66,000 and 990 per cubic millimeter, respectively. Two days after the initiation of treatment, the platelet count rose to 194,000 per cubic millimeter, and 7 percent atypical lymphocytes were found on a peripheral-blood smear. Blood obtained on the day of admission for cultivation of the agent of human granulocytic ehrlichiosis was positive two days after inoculation. A PCR analysis performed on blood obtained at admission also revealed DNA of the agent of human granulocytic ehrlichiosis. Routine blood, urine, and cerebrospinal fluid cultures performed at admission were negative. The child was discharged in good health after five days of intravenous doxycycline. At that time, no morulae were visualized on buffy-coat smears.

An EDTA-treated sample of whole blood obtained from the infant on October 10, five days after birth and three days before her illness, was positive for the agent of human granulocytic ehrlichiosis according to PCR, but negative for morulae. A specimen from that date could not be cultured for the agent of human granulocytic ehrlichiosis because of bacterial contamination. Serologic tests revealed no antibodies to *B. burgdorferi* on October

23, 18 days after birth, but were positive, with a titer of 1:320, for antibodies to the agent of human granulocytic ehrlichiosis. PCR analysis was negative for *B. burgdorferi*. The PCR products of cultured agents of human granulocytic ehrlichiosis from the mother and child had identical restriction-fragment-length polymorphisms.

METHODS

Evaluation for Infection with the Agent of Human Granulocytic Ehrlichiosis

Buffy-coat smears were stained with Wright's stain, and 1000 granulocytes were examined at a magnification of 500 and 1000 for intragranulocytic morulae. PCR testing was performed on EDTA-treated whole blood to detect the agent of human granulocytic ehrlichiosis by the nested procedure of Sumner et al.,³ with the use of primers HSI/HS6 and HS43/HS45. Serologic analysis for antibodies to the agent of human granulocytic ehrlichiosis was performed with an indirect-immunofluorescence assay that used homologous and heterologous Westchester County isolates of the agent of human granulocytic ehrlichiosis cultured in HL-60 cells.⁸ HL-60 cell cultures were performed by adapting the techniques described by Goodman et al.⁹ and were evaluated by Wright's staining for the presence of morulae.^{8,9} The presence of the agent of human granulocytic ehrlichiosis in cultures was confirmed by PCR analysis and an indirect-immunofluorescence assay that used another patient's high-titer antiserum. Restriction-fragment-length polymorphism analysis of the agent of human granulocytic ehrlichiosis was carried out on a 332-bp fragment of the 16S–23S ribosomal DNA intergenic spacer. This region was amplified by species-specific PCR, the resulting prod-

uct was digested with either *HpaI* or *DdeI*, and the digests were analyzed by electrophoresis on 2.5 percent agarose gels.

Evaluation for Infection with *B. burgdorferi*

An ELISA (Wampole Laboratories, Cranbury, N.J.) was used to test for IgG and IgM antibodies to *B. burgdorferi*. Individual immunoblot assays for IgG and IgM antibodies to *B. burgdorferi* (MarDx Diagnostics, Carlsbad, Calif.) were performed and interpreted according to published criteria.¹⁰ PCR analysis to detect *B. burgdorferi* DNA in whole blood treated with an anticoagulant was performed as previously described.¹¹

DISCUSSION

Infection by the agent of human granulocytic ehrlichiosis is an increasing public health concern in the United States and Europe. The agent of human granulocytic ehrlichiosis replicates within granulocytes circulating in peripheral blood, and the case presented here demonstrates that perinatal transmission of human granulocytic ehrlichiosis can occur. The case also sheds some light on the manifestations of clinical human granulocytic ehrlichiosis in the neonatal period.

The mother was apparently infected with the agent of human granulocytic ehrlichiosis toward the end of pregnancy and gave birth to a normal infant. Whether infection with the agent of human granulocytic ehrlichiosis earlier in pregnancy would have had more severe sequelae for mother or child is not known. In sheep and cows, *E. phagocytophila* causes stillbirth or abortion.^{12,13} Recent experiments have shown that *E. phagocytophila* can be transmitted across the placenta in cows.¹⁴ Another ehrlichial species, *E. risticii*, causes abortion and is transmitted transplacentally in horses.¹⁵

Clinical disease caused by the agent of human granulocytic ehrlichiosis has rarely been reported in children.⁷ The reason for this is a matter for speculation. Young sheep and dogs infected with *E. phagocytophila* and an *E. equi*-like organism, respectively, have less severe clinical illness than older animals.^{16,17} However, the infant we describe had a clinical presentation and laboratory abnormalities similar to those found among infected adults,^{7,8} and a very high percentage of this infant's granulocytes were infected with the agent of human granulocytic ehrlichiosis.

The route of infection of the infant could not be determined. The timing of the onset of illness is consistent with all three potential routes of infection (intrauterine, intrapartum, or through breast-feeding). Although it is tempting to speculate that the agent of human granulocytic ehrlichiosis was transmitted transplacentally, this could not be proved, because the umbilical-cord blood and the placenta had been discarded by the time the infant became ill. The sensitivity of PCR analysis of the dried blood from the neonatal blood-spot card is not known, so the negative results cannot be interpreted with confidence. The frozen expressed breast milk was also discarded before we could test it.

Although we suspect transplacental transmission as the route of infection of the infant, we cannot exclude the possibility that secretions containing blood from the birth canal were introduced into the baby through minor skin abrasions or during suctioning of the respiratory tract. *E. phagocytophila* has been found in leukocytes from the milk of cows infected with this organism.¹⁸ However, we believe that transmission of the agent of human granulocytic ehrlichiosis in breast milk is not a likely route of infection in this case because of the small amount of colostrum produced on the first (and only) day of breast-feeding.

The infant's rapid response to the short course of doxycycline is reassuring. However, even short courses of tetracyclines in pregnant women can lead to tooth discoloration in their children. In this case, given the illness of the child and the lack of clinical data on other antibiotics for the treatment of human granulocytic ehrlichiosis, the benefits of using doxycycline appeared to outweigh the risks. Trovafloxacin (a quinolone antibiotic) and rifampin have in vitro activity against the agent of human granulocytic ehrlichiosis¹⁹ but have not been tested in patients with ehrlichiosis. Long-term follow-up of the infant will be required to determine whether human granulocytic ehrlichiosis causes neurodevelopmental problems like those described in human monocytic ehrlichiosis infection of young children.²⁰

The meaning of the mother's positive Western blot assay for IgM antibodies to *B. burgdorferi* is uncertain. She had no erythema migrans rash. Coinfection with the agent of human granulocytic ehrlichiosis and *B. burgdorferi* has been proved by culture of both organisms from samples taken simultaneously from a patient.²¹ However, antibodies alone cannot be used for the diagnosis of Lyme disease in patients acutely infected with the agent of human granulocytic ehrlichiosis, because the production of cross-reactive antibodies is likely.²² In this case, the mother's history of Lyme disease might have increased the likelihood that nonspecific *B. burgdorferi*-reactive antibodies would be produced in the setting of acute disease.

Transplacental transmission of *B. burgdorferi*, which may have devastating consequences in early pregnancy, has been well described.²³ We argue that the agent of human granulocytic ehrlichiosis may also be transmitted by this route. This possibility raises the question of how to treat pregnant women who have had tick bites. In the general (nonpregnant) population, prophylactic antibiotics should not be prescribed routinely after tick bites.²⁴ However, some authorities suggest prophylaxis for pregnant women with tick bites.²⁵ More data are needed to determine the timing and choice of antibiotic for the treatment of pregnant women and newborn infants exposed to or infected by the agent of human granulocytic ehrlichiosis in areas where this disease is endemic.

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