CASE REPORTS

Equine Abortion Associated with the *Borrelia parkeri*-*B. turicatae* Tick-Borne Relapsing Fever Spirochete Group

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Direct amplification and sequencing of the 16S rRNA gene and a variable region of the flagellin gene from fetal liver-associated spirochetes belonging to the *Borrelia parkeri*-*B. turicatae* tick-borne relapsing fever spirochete group with a late-term abortion in a mare are described.

CASE REPORT

A 6-year-old Thoroughbred mare in its ninth month of pregnancy presented with a yellow vaginal discharge. Other than the discharge, the mare appeared clinically normal. A complete blood count was performed; however, all blood parameters were within normal limits. Empirical treatment was initiated and included oral administration of trimethoprim-sulfadiazine and a parenteral nonsteroidal anti-inflammatory medication, flunixin meglumine. After several days of treatment, the mare aborted. Postabortion the mare did not retain her placenta; however, the yellow discharge was still present and persisted for the entire subsequent breeding season (January through June) despite several uterine lavages with isotonic saline followed by intrauterine infusions of either trimethoprim-sulfadiazine, tilmicosin, or ceftiofur sodium. Apart from the discharge, the mare remained clinically normal. The mare manifested normal and regular estrus cycles and was bred to a stallion six times but did not conceive. A uterine biopsy taken 3 months postabortion did not reveal any pathology to account for the reproductive failure. The mare had resided exclusively in the inland foothill area of San Diego County in California during its pregnancy.

Immediately postabortion, the fetus, the entire placenta, and a serum sample from the mare were submitted to the California Animal Health and Food Safety Laboratory-San Bernardino Branch for diagnostic evaluation. The fetus was a female and weighed 17.55 kg, and the distance from crown to rump was 80 cm. Both the fetus and placenta were in fresh postmortem condition. At the time of the necropsy, there was approximately 200 ml of clotted blood in the abdominal cavity. The chorion of the placenta from the nonpregnant horn of the uterus was thickened and corrugated. The serosal surface of the chorion had sparse focal hemorrhages. No other abnormalities were observed. Samples were submitted for microbi-

ologic and histologic examination. A 4-week postabortion convalescent sample from the mare was also submitted for serologic evaluation.

The mare's serum had a titer of 1:64 for equine herpesvirus (EHV)-1 by serum virus neutralization assay and was negative at a 1:4 dilution for equine viral arteritis by serum virus neutralization assay. Both the acute- and convalescent-phase serum samples were negative for antibodies to *Leptospira interrogans* serovars canicola, grippotyphosa, hardjo, icterohemorrhagiae, and pomona at 1:100 by the microscopic agglutination test. A fluorescent-antibody test of fetal liver and lung impressions was negative for EHV-1. Direct fluorescent-antibody testing of a liver impression for *Leptospira* spp. was also negative. The results of attempts at virus isolation by using RK13 and primary equine kidney cell lines were negative.

No organisms were observed on direct Gram stain smears of fetal liver or stomach fluid. Aerobic cultures on 5% sheep blood and MacConkey agars were performed on the fetal liver, lung, and stomach contents and the placenta. No bacteria were isolated from the liver, lung, or stomach contents. The placenta had small numbers of a mixed population of bacteria that were interpreted as contaminants. A culture of stomach contents for *Campylobacter* spp. was negative.

Examination of hematoxylin and eosin-stained sections of routinely fixed and processed samples of the placental chorioallantois, as well as fetal liver, kidney, lung, heart, spleen, and brain, revealed inflammatory lesions in the chorioallantois, liver, and brain. The chorioallantois had multifocal, dense aggregates of degenerate neutrophils (microabscesses) in its base stroma, segmental squamous metaplasia of trophoblastic epithelium, and multifocal infiltration of villous stroma by mononuclear leukocytes. The liver had rare multifocal areas of necrosis and lymphohistiocytic inflammation of parenchyma and portal tracts (Fig. 1A). The brain had generalized endothelial hypertrophy of capillaries and focal-segmental infiltration of mononuclear leukocytes in leptomeninges and perivascular spaces. Silver (Parker modification of Steiner)-stained sections of the placenta, liver, kidneys, and brain revealed the presence of 10- to 15- μ m-long, slender, spiral organisms in all

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FIG. 1. (A) Focal necrosis of liver parenchyma accompanied by a lymphohistiocytic inflammatory response. Magnification, $\times 86$. (B) Silver stain (Parker modification of Steiner stain) of liver demonstrating 10- to 15- μm -long, slender, spiral organisms. Magnification, $\times 858$.

tissues (Fig. 1B). Large numbers of spiral organisms were focally present in the metaplastic placental epithelium and stroma of the chorioallantois, moderate numbers were diffusely present throughout parenchyma of liver and brain, and rare numbers were present in renal cortex. No other organisms were seen in chorioallantois and liver sections stained by Gram (Hucker-Conn), Giemsa (May-Grünwald), and methenamine silver (Gomori) stains. Immunoperoxidase staining of liver for EHV-1 and -4 was negative.

A sample of the fetal liver was fixed in 10% neutral buffered formalin and transferred to modified Karnovsky's (1/2 strength) glutaraldehyde fixative (M. J. Karnovsky, abst. 270 from the 5th Annual Meeting of the American Society for Cell Biology 1965, abstr., *J. Cell Biol.* 27:137A, 1965) prior to postfixation in 2%

osmium tetroxide reduced with 2.5% potassium ferrocyanide (9). Following osmification, the liver was dehydrated through a graded ethanol series and placed into propylene oxide for transition and infiltration into Spurr's epoxy resin formulation. Thin sections were cut, stained with uranyl and lead salts, and viewed through a Zeiss 10C transmission electron microscope at 80 kV accelerating voltage.

Numerous organisms were observed possessing a protoplasmic cylinder with variable numbers of periplasmic flagella surrounded by a ruffled outer membrane, consistent with a spirochetal morphology (Fig. 2). At least 14 periplasmic flagella were observed in some sections; however, the maximum number could not be determined with certainty. The diameters of the cells ranged from 0.17 to 0.26 μm .

FIG. 2. Transmission electron microscopy of thin section of liver from aborted equine fetus showing longitudinal sections and cross-sections of spirochetes. Magnification, $\times 52,500$. Periplasmic flagella are apparent along the protoplasmic cylinder in one section and above the protoplasmic cylinder in another section. The inset shows a cross-section of a spirochete in the fetal liver. Inset magnification, $\times 190,700$. The outer membrane encloses protoplasmic cylinder and periplasmic flagella.

Because of the histopathology and electron microscopy findings, attempts to culture spirochetes were made with fetal liver, kidney, and stomach fluid, although the samples were considered suboptimal because they had been maintained at -20°C . Liver and kidney suspensions and stomach fluid were prepared as 1:10 dilutions in a sterile physiological saline solution. Dark-field examinations of the liver and kidney suspensions revealed spiral-shaped bodies; however, no motility was observed. None of the spiral bodies were observed in the stomach fluid. Samples were inoculated into Ellinghausen-McCullough-Johnson-Harris medium, modified Barbour-Stoenner-Kelley II medium with and without $1\text{ }\mu\text{g}$ of rifampin per ml, and oral treponeme isolation medium (Anaerobe Systems, Morgan Hill, Calif.) with and without $1\text{ }\mu\text{g}$ of rifampin per ml. The Ellinghausen-McCullough-Johnson-Harris medium was incubated at 26°C and other media were incubated at 35°C for one month. A drop from each culture was examined weekly by dark-field microscopy. No spirochetes were recovered from any of the samples.

DNA was obtained from the fetal liver by successive extractions with saturated phenol, phenol:chloroform, and chloroform:isoamyl alcohol. Conserved primers (5'-AGAGTTTGATCCTG GCTCAG-3' and 5'-GGTTACCTTGTTACGACTT-3') for the 16S rRNA gene were used in a PCR to amplify a product of approximately 1,500 bp. The amplified product was cloned into

competent *Escherichia coli* (TOPO TA cloning kit for sequencing; Invitrogen, Carlsbad, Calif.). Both strands of the cloned fragment were sequenced by automated fluorescent cycle sequencing (performed by Davis Sequencing, Davis, Calif.) with primers from conserved regions of the rRNA gene and M13 primers for sites in the cloning vector. A consensus sequence was developed by using multiple overlapping sequences. A BLAST search with the consensus sequence revealed 99.8% homology (1,456 of 1,459 bases) with the 16S rRNA gene for both *Borrelia parkeri* and *Borrelia turicatae*. Phylogenetic analysis was performed, using the Jukes and Cantor model for distance estimation and bootstrap analysis with 1,000 replications, with *Borrelia* sequences obtained from GenBank and the equine fetal liver (EFL) spirochete (10). A neighbor-joining tree that was generated showed that the EFL spirochete clustered with the tick-borne relapsing fever (TBRF) spirochetes, *B. parkeri* and *B. turicatae* (Fig. 3).

A variable region of the flagellin gene of the EFL spirochete was also amplified by PCR from fetal liver DNA as previously described (8). These primers amplify a 266-bp variable region in the central portion of the flagellin gene from a number of *Borrelia* spp. other than *B. burgdorferi*, including the North American TBRF spirochetes (8). The amplified product was sequenced, and an alignment, excluding priming sites, was performed with sequence data obtained from GenBank for *B. parkeri*, *B. turicatae*, and *Borrelia hermsii* and the EFL spiro-

FIG. 3. Phylogenetic tree based on 16S ribosomal DNA sequences of the EFL spirochete and related *Borrelia* species. GenBank accession numbers are shown in parentheses. The numbers above the branches indicate bootstrap values (percentages of 1,000 replications) from a neighbor-joining analysis.

chete (Fig. 4). There was a 100% homology between the EFL spirochete sequence and the *B. parkeri* sequence. The EFL spirochete sequence differed from that of *B. turicatae* by three bases (98.7% homology) and was substantially different from that of *B. hermsii* (89% homology).

Discussion. To our knowledge, this is the first report of an equine abortion associated with one of the TBRF spirochetes. The nonsuppurative inflammation in multiple fetal tissues and the suppurative response in the placenta indicated an infec-

	517	527	537	547	557	567
<i>B. hermsii</i> (M33839)	TTGAGAGTACATGTGGGCGCAAATCAGGATGAGGCAATTGCTGTTAATATTTATGCATCT					
EFL spirochete	--A-----T-----A-----					
<i>B. parkeri</i> (M67461)	--A-----T-----A-----					
<i>B. turicatae</i> (M67462)	--A-----T-----A-----G--					
	577	587	597	607	617	627
<i>B. hermsii</i> (M33839)	AATGTTGCAAATCTTTTTCAGGCTGAAGGCGCTCAGGCTGCTCCAGTGCAAGAGATAGGA					
EFL spirochete	-----C-----T--G--T-T-----CT--G--AGGT-C-					
<i>B. parkeri</i> (M67461)	-----C-----T--G--T-T-----CT--G--AGGT-C-					
<i>B. turicatae</i> (M67462)	-----C-----T--G--T-T-----CT--G--AGGT-C-					
	637	647	657	667	677	687
<i>B. hermsii</i> (M33839)	CAGCAAGAGGAAGGTCAAGCAGCTCCAGCTCCAGCAGCAGCTCCAGCTCAAGGTGGAGTT					
EFL spirochete	--A-----G--T-----T-----A-----					
<i>B. parkeri</i> (M67461)	--A-----G--T-----T-----A-----					
<i>B. turicatae</i> (M67462)	--A-----G--T-----T-----A-----					
	697	707	717	727		
<i>B. hermsii</i> (M33839)	AATTCCCCAATTAATGTTACAACCGCTGTTGATGCTAATA					
EFL spirochete	----A--G-----TA-----					
<i>B. parkeri</i> (M67461)	----A--G-----TA-----					
<i>B. turicatae</i> (M67462)	----T--G-----TA--A-----					

FIG. 4. Alignment of nucleotide sequences for the variable region of the flagellin genes of *B. hermsii*, *B. parkeri*, *B. turicatae*, and the EFL spirochete. The numbers in parentheses after each *Borrelia* species are GenBank accession numbers. The dashes indicate sequence identity with *B. hermsii*. The nucleotide numbering is based on the flagellin gene for *B. hermsii* (8).

tious cause for the abortion. The demonstration of large numbers of spirochetes in affected tissues and the absence of any known abortifacient agent are consistent with a diagnosis of a spirochete-induced abortion. The diagnostic benefit of using silver staining on affected tissues when pathology suggests an infectious process was underscored in this case. Unfortunately, spirochete cultures were not performed at the time of the postmortem examination and tissue samples were held at -20°C afterwards, making recovery unlikely. Although we were unable to obtain an isolate in this case, direct amplification and sequencing of the 16S rRNA gene and a portion of the flagellin gene of the EFL spirochete showed that it belonged in the *B. parkeri*-*B. turicatae* TBRF spirochete group and was most similar to *B. parkeri*.

Borrelia parkeri and *B. turicatae*, along with *B. hermsii*, cause TBRF in humans in North America (4). Identification of TBRF *Borrelia* to the species level is difficult. In the past, designation of a TBRF *Borrelia* species has often been based on geographic location and the associated tick vector. Based on DNA homology studies, it has been proposed that *B. hermsii*, *B. parkeri*, and *B. turicatae* are actually conspecific (3), however, agreement as to the precise taxonomic assignment of these species remains unresolved (6).

Ornithodoros parkeri and *Ornithodoros turicata* are the vectors for *B. parkeri* and *B. turicatae*, respectively. Both of these tick species are found in the western and northwestern United States (2). In California, they are typically found in the central valley and central and southern coastal areas in association with various rodents and lagomorphs and their burrows. The geographic distribution of these ticks includes the area where the mare in this report was kept. Both tick species occasionally feed on humans and other animals (2). The infection in the mare in this report was most likely the result of the mare acting as an incidental host for an infected tick.

TBRF *Borrelia* species in Africa have been associated with high perinatal mortality in humans, as well as identified as a cause of abortion in humans (5). It has been suggested that transplacental transmission is responsible for neonatal spirochetemia. In this report, after infected ticks fed on the mare, a spirochetemia likely resulted. Transplacental transmission would then have allowed for spirochetes to reach the fetus, resulting in an inflammatory reaction in the placenta and fetal tissues and subsequent abortion. This report demonstrates that, although apparently a rare event, abortion can occur in horses infected with TBRF spirochetes. The TBRF spirochetes should be included in the differential diagnosis for equine abortions in areas where tick vectors are known to exist, espe-

cially when no common etiologic agent is identified and a mild to moderate, mixed inflammatory reaction is present. Although the postabortion uterine biopsy in this mare did not show any pathology to indicate chronic reproductive tract damage, a uterine culture 3 months postabortion was positive for *Streptococcus equi* subsp. *zooepidemicus* and *E. coli*, suggesting that a secondary infection was responsible for the subsequent breeding failure. The reproductive soundness of the mare remains unresolved.

Because the Lyme disease spirochete, *B. burgdorferi*, has also been associated with various clinical manifestations in the horse (7), it is important to use appropriate tests to clearly distinguish between infections with that species and TBRF spirochetes. Serologically cross-reactive structural proteins shared by *B. burgdorferi* and TBRF spirochetes suggest that serologic tests alone are not sufficiently specific to be diagnostic (1). In this report, direct amplification and sequencing of the 16S rRNA gene and a portion of the flagellin gene clearly identified the spirochete group involved.

Nucleotide sequence accession numbers. The sequences of the partial flagellin gene and 16S rRNA gene for the EFL spirochete have been deposited in the GenBank database under accession no. AY049948 and AY049949, respectively.

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