Nocardioform placentitis with isolation of Amycolatopsis spp in a Florida-bred mare

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Case Description—A 4-year-old Thoroughbred mare was evaluated because of placental abnormalities and a retained placental remnant.

Clinical Findings—Microbial culture of the placenta yielded pure growth of Amycolatopsis spp. Histologic examination of the placenta revealed a focally expanding chorionitis with intralesional gram-positive filamentous bacilli and multifocal allantoic adenomatous hyperplasia on the apposing allantoic surface.

Treatment and Outcome—Treatment with lavage and oxytocin resulted in expulsion of the placental remnant within hours of parturition. The mare did not become pregnant again despite multiple breedings. The foal appeared healthy but died of complications during an elective surgical procedure at 7 weeks of age.

Conclusions and Clinical Relevance—To the author’s knowledge, all previously confirmed cases of nocardioform placentitis have been in mares bred in the central Kentucky region. Indications that the pathogen in the mare reported here is a different species than that isolated in Kentucky suggest that this is an emerging disease. Mares with nocardioform placentitis usually do not have the same clinical signs as mares with placentitis resulting from an ascending pathogen. (J Am Vet Med Assoc 2006;228:1234–1239)

A 4-year-old Thoroughbred mare from the University of Florida College of Veterinary Medicine teaching herd foaled without complications at 336 days of gestation on April 23, 2004. The mare was artificially inseminated once (May 21) during the first estrous cycle following her foal heat. The sire of the 2004 breeding was a 12-year-old Warmblood stallion owned by a private, local farm. The stallion had lived in Florida for its entire life except for a trip to Kentucky in 1998 for a performance event. Semen for artificial insemination was extended in a commercial, skim milk–based extender except for a trip to Kentucky in 1998 for a performance event. Semen for artificial insemination was extended in a commercial, skim milk–based extender with amikacin sulfate. Microbial culture of the stallion’s raw ejaculate yielded pure growth of Amycolatopsis spp in a Florida-bred mare.

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Conclusions and Clinical Relevance—To the author’s knowledge, all previously confirmed cases of nocardioform placentitis have been in mares bred in the central Kentucky region. Indications that the pathogen in the mare reported here is a different species than that isolated in Kentucky suggest that this is an emerging disease. Mares with nocardioform placentitis usually do not have the same clinical signs as mares with placentitis resulting from an ascending pathogen. (J Am Vet Med Assoc 2006;228:1234–1239)
on the allantoic side of the chorioallantois were multifocal nodules in a pattern radiating from the insertion of the umbilical blood vessels (Figure 2). The amnion and umbilical cord did not have any gross abnormalities. The chorial avillous lesion was compatible with nocardioform placentitis, previously seen at the University of Florida only in mares bred in Kentucky; or placentitis caused by \textit{Cellulosimicrobium cellulans}, which, to the authors’ knowledge, has been reported only in Kentucky. \textsuperscript{1} The amount and nature of the mucopurulent material were also compatible with those differential diagnoses as well as with fungal placentitis.

Because of the retained placental remnant, the mare’s uterus was lavaged with a warm, isotonic saline (0.9% NaCl) solution and the mare was treated with oxytocin (20 units, IM, q 6 h), flunixin meglumine (1.1 mg/kg [0.5 mg/lb], IV, q 12 h), metronidazole (15 mg/kg [6.8 mg/lb], PO, once as a loading dose, then 7.5 mg/kg [3.4 mg/lb], PO, q 6 h), trimethoprim-sulfadiazine (20 mg/kg [9.1 mg/lb], PO, q 12 h), and pentoxifylline (5.0 mg/kg [2.3 mg/lb], PO, q 12 h). During uterine lavage, the placental remnant was identified attached to one of the horns of the uterus; a small weight (approx 250 g) was attached to the placental remnant via umbilical tape. The retained placental remnant was expelled within 12 hours of the estimated foaling time, a few hours after lavage and attachment of the weight. Treatment with uterine lavage (q 24 h) and oxytocin was continued for the next 2 days until intrauterine fluid accumulation had resolved. Treatment with trimethoprim-sulfadiazine was continued for 5 days; all other treatments were discontinued after retrieval of the placental remnant. Other than the accumulation of intrauterine fluid for 2 days, no other clinical signs were detected in the mare.

The foal was found standing and nursing within 2 hours of parturition. The foal was examined and appeared to be clinically normal, except it was small (35 kg [77 lb]; reference weight range for Thoroughbred foals is 45 to 55 kg [99 to 121 lb]). \textsuperscript{1} A sample of blood was submitted for a CBC, and serum was obtained for determination of the serum fibrinogen concentration. Sepsis was suspected in the foal, indicated by lesions seen in the placenta and a high serum fibrinogen concentration (700 mg/dL; reference range, 200 to 400 mg/dL). The foal was treated with ceftiofur (4 mg/kg [1.8 mg/lb], IM, q 12 h) and trimethoprim-sulfadiazine (30 mg/kg [13.6 mg/lb], PO, q 12 h). Eighteen hours after birth, blood was collected and serum was obtained for determination of serum IgG concentration, which was low (<400 mg/dL; reference limit, >800 mg/dL; 400 to 800 mg/dL is considered partial failure of passive transfer). The foal was given 1 L of equine hyperimmune plasma IV. A day after plasma infusion, the foal’s serum IgG concentration was >800 mg/dL. Antimicrobial administration was discontinued after 12 days of treatment.

A sample was obtained from the uterine environment prior to the first uterine lavage and within 12 hours of parturition for microbial culture by use of 5% sheep blood agar plates. Plates were incubated at 36°C in 5% CO\textsubscript{2} for 48 hours; scant growth of \textit{Streptococcus dysgalactiae} \textit{subsp. equisimilis} was detected, which was considered as a contaminant. A sample obtained from the placenta for microbial culture was also plated on 5% sheep blood agar plates, which were incubated first at 36°C for 48 hours and then at 30°C to 33°C for an additional 72 hours; growth of a mixed bacterial flora was detected. Because fungal or nocardioform organisms were suspected, the incubation period was extended. After 5 days of incubation, the predominant bacterium present formed small (1- to 2-mm-diameter), golden-yellow, hard colonies that grew into the agar (Figure 3). A gram-positive, extensively branching, filamentous bacterium was detected that resembled bacteria described as being associated with nocardioform placentitis in horses. \textsuperscript{55} Because this would potentially represent expansion of nocardioform placentitis outside its traditionally associated area (central Kentucky), further diagnostic testing was performed to identify the organism definitively. A pure microbial culture of the bacterium and a frozen piece of the placenta were sent to the Livestock Disease Diagnostic Center at the University of Kentucky for complete analysis of the bacterium by 2 microbiologists (SFS and JMD).

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Figure 2—Photograph of the allantoic surface of the placenta of the foal and mare in Figure 1 (a). Notice the nodular lesions (white arrows) and their location in relation to the attachment of the umbilicus (black arrow). The nodular lesions were diagnosed as allantoic adenomatous hyperplasia. b—Photomicrograph of the chorioallantois from the placenta of the foal and mare in Figure 1. The chorionic surface has complete loss of villi. The allantoic stoma is expanded by hyperplastic epithelium forming pseudo-glands with accumulation of neutrophils and proteinaceous fluid in the lumen. The large vessel between the chorion and allantoic layers is originating from the insertion of umbilical cord vessels into the chorioallantois. H&E stain; bar = 2 mm.
At the Livestock Disease Diagnostic Center, microbial culture of the placenta also yielded an almost pure microbial culture with marked growth of a gram-positive branching bacillus. The bacterium grew better in aerobic than in microaerophilic conditions and did not grow at all in anaerobic conditions. The bacterium grew slowly on blood agar (subcultures required 48 hours of incubation to yield visible growth) and did not grow in anaerobic conditions. The bacterium grew slowly on blood agar (subcultures required 48 hours of incubation to yield visible growth) and did not grow at all in anaerobic conditions. The bacterium was strongly catalase-positive and nonacid-fast by use of the Kinyoun acid-fast stain. The phenotypic characteristics and colony morphology (small, hard, and golden-yellow colonies) were consistent with bacteria isolated from cases of nocardioform placentitis of the genus *Amycolatopsis*.

To determine whether the case reported here represented an expansion of the same *Amycolatopsis* spp indicative of cases of placentitis seen in Kentucky (and therefore a possible geographic expansion in the range of the pathogen) or whether this was a new species (potentially representing a new, emerging pathogen), genomic sequencing was pursued. Genomic DNA from the bacterium was isolated, purified, and sequenced; data were assembled and edited following procedures described elsewhere.

Results of similarity searches by use of the Basic Local Alignment Search Tool (BLAST) provided by the National Center for Biotechnology Information indicated that the bacterium had the closest similarity to the following 4 species: *Amycolatopsis vancoresmycina* (GenBank accession No. AJ508240), *Amycolatopsis kentuckyensis* (GenBank accession No. AY183337), *Amycolatopsis rifamycinica* (GenBank accession No. AY083603), and *Amycolatopsis tolypomycina* (GenBank accession No. AJ508241). The similarity to all 4 species was >99%. Greater than 1,400 base pairs were compared, and differences were detected in only 4 to 14 sites. Except for *A. kentuckyensis*, the other species listed above have been recovered from soil samples and produce antimicrobials.

*Amycolatopsis kentuckyensis* was 1 of 3 species described by Labeda et al. that had been isolated from placentas from horses with nocardioform placentitis. Results of other studies indicate that type strains of *Amycolatopsis* spp with >98.5% similarity in the 16S rRNA gene sequence are different species. Therefore, the bacterium isolated from the placenta from the mare reported here may have been a new species, which could have been determined only by DNA reassociation studies.

Histologic examination of the placenta revealed a focally expanding chorionitis with intralesional gram-positive filamentous bacilli and multifocal allantoic adenomatous hyperplasia on the apposing allantoic surface. The expanding edge of the lesion had villus infiltration by neutrophils with villus edema, necrosis, and eventual sloughing. Sloughed necrotic villi were surrounded by an amorphous eosinophilic material, and neutrophils coated the remaining villar stumps (Figure 4). Moderate numbers of filamentous gram-positive bacilli were detected in superficial debris and scattered between degenerating trophoblastic epithelial cells (Figure 5). The remaining villi were blunted and...
fused and had stromal infiltration by lymphocytes, macrophages, and neutrophils. The more chronic central areas of the lesion had complete loss of villi and mild, diffuse infiltration of primarily lymphocytes throughout the adjacent chorionic stroma. The allantoic side of the chorioallantois, often adjacent to large umbilical cord–associated vessels, had multifocal nodular allantoic adenomatous hyperplasia. The adenomatous hyperplasia was composed of hyperplastic allantoic epithelium that was forming pseudoglands filled with neutrophils and proteinaceous fluid. Coalescing pseudoglands of various sizes were markedly expanding the allantoic stroma to form grossly visible nodules. The remaining chorioallantois, amnion, and umbilical cord had mild to moderate diffuse congestion.

The mare was bred on the cycle after the foal heat; however, transrectal ultrasound evaluation of the uterus 14 days later revealed that the mare was not pregnant, although a marked amount of uterine edema was detected. Microbial culture of a sample from the uterus was repeated and resulted in scant growth of Klebsiella spp. The mare was treated with an intravenous infusion of gentamicin (1 g of gentamicin diluted with 40 mL of an 8.4% sodium bicarbonate solution, q 24 h) for 5 days. Microbial culture of a sample from the uterus after treatment resulted in scant to moderate growth of Escherichia coli. Intravenous lavage was performed by use of a 1% povidine iodine solution once daily for 4 days. A uterine swab specimen was submitted for aerobic microbial culture during the next estrous cycle; no growth was detected. The mare was bred via artificial insemination with good-quality semen from a proven stallion 5 additional times during the breeding season after the foal heat; no growth was detected. The mare was not suspected of having placentitis and therefore was not receiving any medical treatments. However, in retrospect, transabdominal ultrasonographic evaluation was indicated and ultrasonographic signs of placentitis may have been detected that would have warranted appropriate treatment.

Most mares affected by nocardioform placentitis do not require subsequent treatment and do not have clinical signs of infertility the following breeding season. The mare of this report may have developed uterine inflammation in response to antimicrobial treatment, such as administration of antimicrobial, anti-inflammatory, and, in some cases, tocolytic drugs. The mare of this report was not receiving any medical treatments. However, in our report truly is a new species, this may be an indication of an emerging disease as opposed to an expanding one.

Nocardioform placentitis often results in abortion or premature delivery. Prior to foaling or abortion, mares with nocardioform placentitis may have premature mammary gland development but will not have vulvar discharge, which is attributable to the location of infection in the body of the uterus, away from the cervical area. Other differential diagnoses for this clinical finding include twins and placentitis caused by C. cellulans. The possibility of twins must be differentiated from infectious etiologies via transabdominal ultrasonography. Both nocardioform and C. cellulans infections may cause ultrasonographic signs of placentitis, potentially including placental thickening, placental detachment, increased amniotic or allantoic fluid cellularity, or change in fetal heart rate. Cellulosimicrobium cellulans infections may or may not cause ultrasonographic signs of inflammation visible via transrectal ultrasonographic examination. Clinical signs associated with nocardioform placentitis will not be visible via transrectal ultrasonographic examination. Transabdominal ultrasonography in late-term mares with any abnormal or premature clinical signs (eg, mammary gland development) is routinely used as a diagnostic tool by many equine practitioners in the central Kentucky region to scan for signs consistent with nocardioform or C. cellulans placentitis. Ultrasonographic confirmation of clinical signs consistent with placentitis should be followed by appropriate medical treatment, such as administration of antimicrobial, anti-inflammatory, and, in some cases, tocolytic drugs. The mare of this report was not suspected of having placentitis and therefore was not receiving any medical treatments. However, in retrospect, transabdominal ultrasonographic evaluation was indicated and ultrasonographic signs of placentitis may have been detected that would have warranted appropriate treatment.

Discussion

Nocardioform placentitis is a leading cause of reproductive losses in horses bred in central Kentucky and has been reported in Europe and South Africa. The mare reported here had been housed exclusively in Florida and bred via artificial insemination by use of fresh, chilled semen from a Warmblood stallion, also housed exclusively in Florida. All other mares on the farm, except one, had been together on the farm for at least 2 years prior to the 2004 foaling season. The new mare was pregnant when purchased as a pregnant animal from a local horse dealer, and the origin of that mare was not known, although shefoaled without any clinical signs of placentitis. To our knowledge, this is the first report of nocardioform placentitis in a horse in the United States that was not associated with the central Kentucky region. Nocardioform placentitis has been diagnosed in mares in other regions of the United States; however, they had been bred in the central Kentucky region before moving to another state for the duration of their pregnancy. If the bacterium described in our report truly is a new species, this may be an indication of an emerging disease as opposed to an expanding one.
Although the pathogenicity of most and placental insufficiency is not known.

The sire of the foal in this report was a Warmblood stallion. All other mares on the farm had been bred by a Thoroughbred stallion housed on the farm, which was slightly smaller than the Warmblood stallion. The foal was much smaller and remained smaller than all other foals of similar age. Growth of foals born to mares with placentitis has been found to be stunted. The small size of these foals is thought to be attributable to sepsis or placental insufficiency. A strong correlation between foal birth weight and the total microscopic area of contact at the placental interface has been detected. In the foal of this report, there were early clinical signs of neonatal sepsis and there was reason to suspect placental insufficiency given the large, avillus chorioallantoic lesion.

Allantoic adenomatous hyperplasia has been closely associated with placentitis. Hong et al reported a close association between the formation of allantoic adenomatous hyperplasia and other placental pathologic lesions, including placentitis, placental edema, and fetal diarrhea. In that report, 9 of 63 horses with adenomatous hyperplasia also had pathologic lesions and bacterial identification compatible with nocardioform placentitis. Histologic lesions were classified into 3 stages. The first 2 stages involved only histologic changes, including complete loss of chorionic villi and hyperplastic epithelium forming pseudoglands in the allantoic stroma with accumulation of neutrophils and proteinaceous fluid in the lumen; all of these changes were detected in the placenta from the mare reported here. Only the most severe, advanced stage (stage 3) had gross lesions, as were detected in the placenta from the mare reported here. Most often, these visible nodular masses were located near the insertion of the umbilical blood vessels, as was also detected in the placenta of the mare reported here. Hong et al suggest that these hyperplastic allantoic lesions may be secondary to chronic irritation, and, therefore, that these lesions would be associated with primary placental pathologic conditions, especially placentitis, and absent in conditions such as umbilical torsion, twinning, or death attributable to dystocia.

Nocardioform organisms isolated from placentas of horses and identified by determining the sequence of the 16S rRNA gene include species in the genus Amycolatopsis and Crossiella equi. Gram staining of tissues infected with nocardioform organisms reveal filamentous, branching, gram-positive bacteria invading degenerating trophoblast cells and in mucopurulent and cellular debris adhered to the surface of the chorion. Isolates from these groups of bacteria are not commonly detected in veterinary diagnostic laboratories, and procedures for their identification by use of phenotypic characteristics are rarely available or used. Identification by analyzing the 16S rRNA gene presently remains the best way to identify these bacteria.

The pathogenesis of nocardioform placentitis is unresolved. Traditionally, we regard 2 main routes of placental infection: ascending via the cervix and hematogenous spread. The former route results in loss of chorionic villi around the cervical star, whereas the latter route has a more generalized, diffuse loss of villi. The lesion classically detected in mares with nocardioform placentitis is not compatible with either of these descriptions. Placentas from mares with nocardioform placentitis, similar to the mare reported here, classically have a loss of chorionic microvilli in a focal area of the placenta associated with the base of the uterine horns. It has been postulated that the pathogen is present or introduced in the mare at the time of breeding and settles in the ventral aspect of the uterus, where it causes infection. This hypothesis has not been proven, although it is likely that nocardioform organisms are from the environment. Reportedly, various nocardioform organisms have been detected in the soil in other countries, although the pathogenicity of most of these organisms has not been determined. Although microbial culture of semen from the Warmblood stallion was performed during the same breeding season and did not yield any pathogens, microbial culture of a sample from the stallion’s penis was not performed; therefore, the stallion cannot be ruled out as the source of the infection. It seems unlikely that the infection would ascend via the cervix after establishment of the fetal-placental unit because microvilli surrounding the cervical star remain unaffected in these mares. The avillus pattern caused by nocardioform organisms also does not fit the classical pathogenesis of hematogenous lesions because nocardioform organisms cause a focal lesion, usually located at the base of the uterine horns.

Nocardioform placentitis has been a major cause of placentitis and reproductive losses in the central Kentucky region for 20 years. It has been associated with mares bred in central Kentucky (1 case of nocardioform placentitis was reported in South Africa in 2001, and 1 was reported in Italy in 2004). Similar cases of nocardioform placentitis not associated with mares bred in central Kentucky have been confirmed by other diagnostic laboratories, including a mare and stallion in Florida and a mare and stallion in Virginia. It is possible that this notable group of pathogens, which in the United States has until recently been confined to central Kentucky, will emerge as an important cause of pregnancy loss in horses in other areas of the country. Nocardioform organisms are soil-borne and cosmopolitan in distribution. Equine practitioners need to be vigilant in examining placentas from premature or aborted foals and may need to adopt measures currently considered as common practice in central Kentucky (eg, performing transabdominal ultrasonography routinely in late-term mares suspected of having placentitis), so that these unique cases of placentitis may be detected before abortion occurs.

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