

MESSAGE FROM THE GRAYSON-JOCKEY CLUB RESEARCH FOUNDATION

RESEARCH UPDATE: NOCARDIOFORM PLACENTITIS

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Nocardioform placentitis remains an enigmatic and poorly understood disease that was first diagnosed in central Kentucky in 1986. Since that time, nocardioform placentitis has occurred episodically with outbreaks in 1998, 1999, 2011, 2017 and 2020.

Cases of nocardioform placentitis have also been reported in South Africa, Italy, Australia, and New Zealand with anecdotal reports of the disease in eight other U.S. states during 2020. Nocardioform placentitis is characterized by late-term abortions, premature foals, neonatal deaths, and weak, stunted foals at term associated with an apparent fetal growth retardation due to placental insufficiency.

The resulting placental insufficiency and fetal growth retardation are related to the size of the lesion on the placenta. The distribution of the placental lesion is distinct from ascending bacterial placentitis with lesions of nocardioform placentitis distributed along the bottom portion of the placenta near the junction of the uterine horns and body. The lesion is often sharply demarcated from the surrounding normal placenta, and the affected placenta is covered with a thick, tan mucoid material. Although the placenta shows inflammatory changes, the infection with nocardioform placentitis seems limited to the placenta and does not involve the fetus in contrast to other forms of bacterial placentitis.

Nocardioform placentitis is associated with gram-positive, branching actinomycetes including *Amycolatopsis spp.*, and *Crossiella equi* along with more recently characterized isolates of *Streptomyces atiruber* and *Streptomyces silaceus*. Characterization of actinomycetes during the 2020 outbreak of nocardioform placentitis in Central Kentucky revealed that *Amycolatopsis spp.* was most common with *Crossiella equi* as the next most frequent isolate.

The ecology of these bacteria remains poorly understood as they have only been isolated from placental lesions and not from environmental sources. Attempts to induce the infection in mares by intrauterine inoculation of *Crossiella equi* at breeding and in pregnant mares via oral, intravenous, and intranasal routes have been unsuccessful. Therefore, the pathogenesis of nocardioform placentitis remains obscure.

Nocardioform placentitis abortions occur between November and June with a peak incidence in January, February, and March. The majority of affected fetuses are in the last trimester of pregnancy, and the identification of nocardioform lesions on the placenta of term pregnancies is a common presentation. Retrospective evaluation of monthly climate data (precipitation and average temperature) for Central Kentucky and the number of nocardioform placentitis cases seen by the University of Kentucky Veterinary Diagnostic Laboratory for the period between 1990-2020 was conducted. This analysis demonstrated a moderately strong negative association ($\rho = -0.60$; $P = 0.0004$) between August and September rainfall and the number of nocardioform placentitis cases submitted for the subsequent foaling season. Likewise, there was a moderately strong positive association ($\rho = 0.52$; $P = 0.003$) between mean temperatures in August and September and the number of nocardioform placentitis cases submitted in the subsequent foaling season. The strong association between a hot and dry August and September preceding

an increase in nocardioform cases is strongly suggestive of an environmental factor in this disease.

It is worth considering that the actinomycetes responsible for nocardioform placentitis might originate as soil-born organisms, despite the failure thus far to isolate these organisms from environmental sources. Related members of *Amycolatopsis* and *Streptomyces* are well characterized as soil-associated microorganisms.

Clinically, diagnosis of nocardioform placentitis before foaling remains difficult. Late in the disease, affected mares might show signs of premature lactation and mammary development but typically do not show a vulvar discharge. Evaluation of the uterus and placenta by transrectal ultrasound exam is often normal unless the disease has progressed extensively. Imaging the lesion with nocardioform placentitis requires examining the pregnant uterus with ultrasound through the body wall, a time consuming and diagnostically challenging procedure.

The true incidence of nocardioform placentitis remains unknown. Although cases with severe neonatal outcome (dead or very poorly developed neonates) are likely submitted to the UKVDL for pathologic workup, births that have lesions of nocardioform placentitis without effects on the fetus are much less likely to be submitted and remain uncounted.

This is supported by careful screening of all placentas on one farm that revealed frequent and often small lesions consistent with nocardioform placentitis without obvious neonatal effects during the 2020 foaling season. Accurately determining the impact of nocardioform placentitis is further complicated by incorrect diagnosis of the disease on farm.

During the 2020 outbreak, 171 placentas from affected mares and normal control mares were evaluated. Upward of 25% of the submitted placentas were incorrectly identified on the farm as normal or as nocar-

dioform placentitis, which emphasizes the importance of submitting these materials to a veterinary diagnostic lab for definitive diagnosis.

During the 2020 outbreak of nocardioform placentitis, a variety of clinical, epidemiologic, and pathology findings were generated from 264 pregnant mares with either nocardioform placentitis or normal control mares. Highlights from these data indicate that mares with nocardioform placentitis were older, had shorter gestational lengths, more dead neonates, and reduced foal birthweights compared to control mares without placentitis. Furthermore, neonatal weights were related to the size of the placental lesion (larger lesions had smaller neonates and were more likely associated with dead neonates). Interestingly, mares with nocardioform placentitis appeared to have no reduction in their fertility after foaling compared to controls.

The underlying etiology of nocardioform placentitis remains unknown despite the disease's being first identified more than 30 years ago. It appears likely the pathogenesis of nocardioform placentitis is mul-

tifactorial with a strong environmental association (hot, dry periods in late summer preceding the next foaling season). A number of related bacteria are isolated from nocardioform placentitis lesions, and many of these bacteria are from groups that are often associated with soil. This suggests that there is likely some upstream, common environmental factor involved with the development of nocardioform placentitis. The nature of this factor remains speculative.

Ongoing studies at the Gluck Center funded by the Grayson-Jockey Club Research Foundation, the Koller Priority Response Fund, and the Albert G. Clay Endowment are examining multiple aspects of this disease. These studies will examine possible markers in blood of pregnant mares, including steroid hormones, cytokines, alpha-fetoprotein and transcripts for inflammatory signaling.

Other studies will examine the role of the mare's immune response and antibody formation to bacteria associated with nocardioform placentitis. Data collected during the 2020 outbreak will also be used for a more detailed epidemiologic investigation of the

disease. High-throughput RNA sequencing will be used to examine the interaction of the pathogenic bacteria with the placenta in order to understand how these organisms induce disease and the response of the mare to the infection. Work is also underway to develop better in vitro models for the disease, which to date has not been successfully induced experimentally.

These studies will provide a better grasp of the causes, outcomes and possible preventions and treatments for this enigmatic disease. **BH**

In September 2020, a half-day virtual workshop on nocardioform placentitis was held at the University of Kentucky. Presentations from this workshop and the associated white paper can be found at this web address:

<https://gluck.ca.uky.edu/nocardioform-sept2020>

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