2020

The Virtual Workshop on Nocardioform Placentitis

WORKSHOP LEADER

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Agenda (Date: 9-28-2020)

Time	Speaker	Topic title	Link to recorded video
Noon	Dr. Barry A. Ball	Welcome and introduction	
12:15 pm	Dr. Jackie Smith	UKVDL Nocardioform placentitis for 2020 Foal Crop.	https://youtu.be/cd5MealG8Ko?list=PL1SaMrhjACARAz0IPoYGRsZSXm99bH7od
12:30 pm	Dr. Rebecca Ruby	Pathology of Nocardioform placentitis 2020	https://youtu.be/Mo7ebgZX8x0
12:45 pm	Dr. Erdal Erol	Microbiology of Nocardioform Placentitis in Horses	https://youtu.be/JLRKfNi3r30
1:00 pm	Dr. Kristina G. Lu	Clinical disease	https://youtu.be/dfjX9W0Kkt0
1:15 pm	Dr. **Maria Schnobrich**	Clinical disease	https://youtu.be/5YApEqeIXjo
1:30 pm	Dr. Scott Bailey	On farm experience with Nocardioform placentitis in 2020	https://youtu.be/pqKeBDtddGQ
1:45 pm		**Discussion and break**	
2:00 pm	Dr. Carleigh Fedorka	2020 Nocardioform placentitis - epidemiologic information	https://youtu.be/SqeMUObsttw
2:15 pm	Dr. Hossam Elsheikh Ali	Transcriptomics of the placenta with Nocardioform placentitis	https://youtu.be/J-aDuTHkeYk
2:30 pm	Dr. Pouya Dini	Host-pathogen sequencing in NP	https://youtu.be/tPTe-ZCwoXI
2:45 pm	Dr. Barry A. Ball	Climate associations and outcomes	https://youtu.be/zPPCRitV0aU
3:00 pm		**Discussion and break**	
3:15 pm	Dr. Carrie Shaffer	Possible generation of in vitro placental models	Not Available
3:30 pm	Dr. Allen Page	Planned research - serology and peripheral cytokine mRNA expression	https://youtu.be/Gx1j76fk44c
3:45 pm	Dr. Judy CAWDELL-SMITH	Serendipity – unexpected research findings relevant to Nocardioform placentitis	https://youtu.be/ADVnWZVxNow
4:00 pm	Dr. Mats H. T. Troedsson	Proposed research regarding Nocardioform placentitis	https://youtu.be/j6LD7MBQLBw
4:15 pm -		**Discussion and summary**	

Updates on Equine Nocardioform Placentitis

Hossam El-Sheikh Ali, Carleigh Fedorka, and Barry Ball

Nocardioform placentitis (NP) continues to result in episodic outbreaks of abortion and preterm birth in mares and remains a poorly understood disease. This workshop aimed to cover a range of relevant topics on this enigmatic disease with a special focus on the results to date from the 2020 foaling season, ongoing research, and future research needs related to this problem.

N.B. The underlined text highlights the most recent updates about this disease.

History and outbreaks:

- Nocardioform placentitis (NP) was first diagnosed in central Kentucky (KY) in 1986 with subsequent epizootics occurring in 1998, 1999, 2011, 2017, and 2020.
- During the 2020 outbreak, 207 NP cases were submitted to UK-VDL. This number of cases eclipsed the 2017 outbreak but was empirically lower than the 2011 outbreak.
- Cases of NP have also been reported sporadically in Florida, South Africa, Italy, and most recently in New Zealand.
- Personal communications with nationwide veterinarians revealed that NP cases have also been reported sporadically in Ohio, Maryland, Texas, California, New York, North Carolina Indiana and Pennsylvania. Additionally, some of these cases were recorded in Standardbred mares in Ohio, and Pennsylvania.

Etiology:

- Nocardioform placentitis is associated with gram-positive, branching actinomycetes, including *Amycolatopsis* spp., and *Crossiella equi* along with more recently characterized isolates of *Streptomyces atriruber* and *Streptomyces silaceus*, among others.
- Characterization of actinomycetes associated with the 2020 NP outbreak in central Kentucky revealed that *Amycolatopsis spp*. (35.05 % of cases) was the most common, with *Crossiella equi* (16.49 % of cases) as the next most frequent isolate.
- In the 2020 NP outbreak, 45.36 % of cases of mucoid placentitis were not positive for either *Amycolatopsis spp*. or *Crossiella equi.* This might address the possibility that other bacteria are involved in mucoid placentitis and/or the difficulty of detecting bacteria following antimicrobial treatment.
- A preliminary 16s Metagenomic sequencing revealed that focal mucoid placentitis could be associated with other bacterial genera such as *Cellulosimicrobium*, *Trichococcus*, among others.
- Crossiella equi infections may be more likely to result in abortion (severe disease), whereas infections with other type actinomycetes tend to result in live but premature foals
- Although Actinomycetes is a group of common soil microorganism, attempts in 2011 to culture *Amycolatopsis spp.* and *Crossiella equi* from Central Kentucky (KY) soil samples failed.

Pathogenesis/transmission of infection:

- Not yet identified
- The distribution of the NP lesion in the placenta is distinct from ascending, diffuse (hematogenous), and multifocal placentitis with lesions of NP localized in the cranial-ventral portion of the placenta near the junction of the uterine horns and body.
- The site and solitary nature of NP lesion do not fit with ascending or hematogenous bacterial infection.
- 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 with *Crossiella equi* were unsuccessful.
- In Australia, experimental trials to induce equine amnionitis and fetal loss (EAFL) through feeding mares with processional caterpillar resulted in unexpected cases of nocardioform-like focal mucoid placentitis. Therefore, the possible implication of caterpillar in NP should not be neglected.

Possible predisposing factors and occurrence:

- The disease is likely multifactorial and may involve factors such as host factors and environmental conditions.
- Interestingly, the NP epizootic outbreaks in central KY were usually preceded by dry and hot weather in August and September in 2010, 2016, and 2019.
- The previous observation might propose the role of environmental conditions as a strong predisposing factor for the disease.
- The incidence of NP is higher in statistically older mares.
- The method of breeding does not appear to be a factor in NP. For example, NP has been reported with live cover, artificial insemination, and embryo transfer.
- Large farms with higher stocking density are more at-risk.
- Nocardioform placentitis tends to manifest during the last trimester of pregnancy, and abortions occur between November and June, with the highest incidence in January and February.

Lesion:

- Size:
  - Variable (range; 5 X 3 cm – 100 X 50 cm).
  - Very small lesions could be missed during the routine postpartum evaluation of the placenta.
  - Increased lesion size is associated with growth retarded or dead foal .
  - There is a negative correlation between the lesion size and the foal weight (i.e., the larger the lesion, the smaller the foal and vice versa).
- Location:
  - Variable but mainly in the cranial-ventral portion of the placenta near the junction of the uterine horns and body.
  - In 2020 Outbreak, six lesions (cases) involved the cervical star region.

- Gross Appearance:
  - The lesion is often sharply demarcated from the surrounding normal placenta and is with covered thick, tan mucoid material.
  - Apparently, the bacterial infection starts at the center of the lesion and then progress/expand outward.
  - The center of the lesion is most commonly avillous (complete loss of chorionic microvilli).
  - At the margin of the lesion, we can see raised, irregular and red areas of the chorion.
  - N.B: the margin of the lesion is the best sampling site for bacterial culture, PCR, and histopathology.
  - Multifocal lesions were noted in a small subset of cases.
  - Cystic adenomatous hyperplasia on the allantoic surface has been reported in association with NP.
- Exudate:
  - The lesion is usually covered with a characteristic thick, tan mucoid material.
  - Variation in coloration and consistency could be seen among different NP cases.
  - Some cases might not have this characteristic mucoid exudate (i.e., avillous chorion with no mucoid exudate). But will have a similar exudate evident microscopically. This might be attributed to disease chronicity or treatment.
- Histopathology:
  - Center of the lesion: the chorionic villi are blunted and atrophied with lymphocytic infiltrates.
  - The margin of the lesion: the chorioallantois may demonstrate the infiltration of neutrophils, lymphocytes, and plasma cells with squamous metaplasia, blunting and loss of the of the chorionic microvilli. The surface exudate contains sloughed epithelial cells, leukocytes, and an eosinophilic, amorphous material.

Pathobiology:

- The nocardiofrom bacterial infections limited to the chorioallantois (i.e., the amnion and the fetus are not affected).
- In the 2020 NP outbreak, one foal was affected with hepatitis and cultured positive for gram-positive, branching actinomycetes (not confirmed if it is Amycolatopsis spp.or Crossiella equi).
- In the 2020 NP outbreak, one NP case showed abnormalities on the amniotic membrane.
- NP is associated with placental inflammation and separation with consequent placental insufficiency, which in turn affects fetal growth and/or pregnancy outcome (abortion or preterm birth).
- Placental insufficiency is related to aberrant expression of several placenta-regulatory genes and dysregulation of placental angiogenesis, and nutrient transporters, as well as placental hypoxia.
- Placental separation is related to the upregulation of matrix metalloproteinase and apoptosis-related genes.

- No information is available about the endometrial changes during NP.

Outcomes:

- Pregnancy outcomes might be dependent on several factors (size of the lesion, severity of inflammation, causative bacteria, …., etc.).
- Late term-abortion, weak yet viable foals, or normal foal delivery.
- Decreased gestational length (10 days less than unaffected mares) and decreased neonatal weight (10 lb less).
- While neonates may be smaller, they do not have abnormal or altered IgG or WBCs
- NP lesions may also be seen in the CA in mares with normal neonates.
- Normal postpartum fertility, with an average of 1.5 cycle per pregnancy.

Diagnosis:

- Clinical signs:
  - Premature mammary gland development (usually associated with large lesions, which might reflect a late stage of the disease).
  - Vulvar discharge is not commonly observed unless the mare is about to abort.
- Ultrasonography:
  - Transabdominal ultrasonography: to detect placental separation (i.e., separation of the chorion from the endometrium) and accumulation of hyperechoic exudate. Also, CTUP could be measured and compared to the normal reference range.
  - Lesions might be missed during scanning trans-abdominal ultrasonography (e.g., small lesions or very limited area of the uterus is precisely visualized by transabdominal ultrasonography).
  - Special attention should be given to the uterine bifurcation area.
- Blood biomarkers:
  - Abnormal estrogen and progesterone profiles have been reported with NP.
  - No sensitive and specific biomarker is available yet.
- Intrapartum and Postpartum Findings:
  - Late term-abortion or preterm birth.
  - Characteristic NP/focal mucoid placentitis lesion, as mentioned earlier.
  - Obtaining swabs to culture and isolate nocardioform actinomycetes on blood agar.
  - Using culture isolates to run PCR for actinomycetes (i.e., Crossiella equi and Amycolatopsis ssp).

    N.B. The primers used for Amycolaptopsis spp are designed to identify Genus Amycolaptopsis, not the species-specific Classical features of an aborted fetus (decreased body weight/smaller than normal, lack of internal adipose tissue, and decreased musculature).

Treatment:

- Treatment protocols vary by practitioner and severity of disease, but tend to include broad spectrum antimicrobials, anti-inflammatories, tucolytics, and immunomodulators.
- However, this treatment regimen is empirical, and the importance of each treatment component has not been critically evaluated.

- Treatment outcomes might be dependent on several factors (stage of the disease/size of the lesion, causative bacteria, gestational age,…etc.).
- *In vitro* studies revealed that nocardioform actinomycetes are sensitive to ceftriaxone, doxycycline, minocycline, linezolid, and trimethoprim/sulfametaxazole. Further studies to investigate the placental diffusion of antibacterial treatments are warranted.

Ongoing research:

- Epidemiology of 2020 NP
- Dual RNA-sequencing to understand the pathophysiology and the host-pathogen interaction in NP.
- qRT-PCR to measure the inflammatory response in whole-blood mRNA (mainly WBCs) to identify potential biomarkers for nocardioform placentitis
- Serum proteomics and lipidomics to identify potential biomarkers.
- 16s metagenomic sequencing of equine chorioallantois from several NP cases to identify other bacteria associated with the disease.
- Transcriptomics analysis of equine chorioallantois from NP cases caused by Crossiella equi, which is more likely associated with abortion.

Questions and Future Research:

- Evaluation of the pathogenicity of Nocardioform actinomycetes
- Environmental screening of nocardioform actinomyces, using shotgun metagenomic sequencing, to identify the source of these bacteria.
- *In vivo* studies to evaluate the placental diffusion of ceftriaxone, doxycycline, minocycline, linezolid, and TMP/SMZ as a possible antibacterial treatment.
- Trials to develop an equine model and/or experimental animal model for nocardioform placentitis
- Development of equine placental organoids to study nocardioform placentitis (e.g., molecular mechanisms underlying the disease, host-pathogen interaction, bacterial pathogenicity, treatment trials).
- Development of a screening ELISA (bacteria-based ELISA) for Amycolatopsis spp. and Crossiella equi

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Sources and Further Readings [1-15]

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