Horse Species Symposium: Pathogenic and Reproductive Dysfunction in Horses

1095 Monitoring pathogen progression during uterine infection in the mare using biophotonic imaging technology and *lux*-modified bacteria. P. L. Ryan*, D. L. Christiansen, R. M. Hopper, F. K. Walters, K. Moulton, J. Curbelo, and S. T. Willard, *Mississippi State University*.

Premature birth is the leading cause of prenatal morbidity in humans with an incidence of 12.5% (Berhman, 2006). Moreover, 40% of premature births may be attributed to antenatal infection (Lettieri et al., 1993) of which *Escherichia coli* (*E. coli*) has been identified as the most common organism isolated from pregnant women (Lavanya and Jogalakshmi, 2002). In cattle, the rate of uterine infection has been estimated to be from 2.2 to 37.3% (Kelton et al., 1998) with *E. coli* being the most common isolated bacteria in cows with postpartum uterine infections. Similarly, placental infection due to opportunistic pathogens such as *Streptococcus equi* subspecies *zooepidemicus* and *E. coli* are common causes of abortion, still birth and premature delivery in horses (Giles et al., 1993). Moreover, pathogen progression during uterine infections and placentitis may involve invasion of endometrial and fetal tissues, including the brain, leading to increased pro-inflammatory cytokine expression resulting in onset of premature delivery and/or fetal neurological damage (LeBlanc et al., 2002). However, little is known about bacterial pathogenesis during uterine infections in the equid species. Recently, transgenically modified bacteria transformed with the pAK1-*lux* plasmid have been utilized to better understand pathogen progression in the late-term pregnant ewe and mare. A Peltier cooled slow scan CCD camera and a XR/MEGA-10Z bioluminescence imaging camera were employed in these studies to detect *lux*-expressing bacteria. This presentation will explore the use of biophotonic imaging technology and *lux*-modified bacteria to better understand pathogen progression and invasion during uterine infections in pregnant mares, and rate of pathogen clearance postpartum following therapy. The data will demonstrate that bioluminescence and real-time imaging provide a novel means of understanding pathogenesis of bacterial-induced placentitis and preterm birth in horses. Application of this novel imaging technology with *lux*-modified organisms may facilitate the development of more targeted therapeutic interventions.

Key Words: uterine infection, biophotonic imaging, mare

1096 Contagious equine metritis: An insidious threat to the US horse breeding industry. P. J. Timoney*, *Maxwell H. Gluck Equine Research Center*, *Lexington, KY*.

The objective of this presentation is to assess the significance of contagious equine metritis (CEM) as a threat to the economy of the US horse industry.

CEM has given rise to international concern since it was first recognized as a novel venereal disease of equids in 1977. Initial reports highlighted the dramatic clinical features of the disease and how readily transmissible it was. The etiologic agent was identified as a previously undescribed bacterium, *Taylorella equigenitalis*. International concerns over CEM centered on the ease with which this bacterium could be disseminated, the significance of *Taylorella equigenitalis* as a cause of short-term infertility in the mare and existence of the carrier state in the stallion and the mare. The first known outbreak of CEM in the USA was in Kentucky in 1978. The economic impact on the state′s TB industry was substantial. Prior to 2008, additional small-scale outbreaks occurred in Missouri in 1979, Kentucky in 1982 and Wisconsin in 2006, all traced to the importation of carrier animals. On each occasion, appropriate measures were taken to eliminate the infection, resulting in the US regaining its CEM-free status. With the exception of the 1978 occurrence in Kentucky, none of the subsequent outbreaks impacted significantly on the horse industry. That changed dramatically in 2008 after discovery of a culture positive QH stallion in Kentucky. Subsequent investigations turned up 22 carrier stallions and 5 carrier mares in 8 states. Shipment of infective semen and indirect venereal contact in stallion collection centers were major factors in the spread of *Taylorella equigenitalis*. Trace-back investigations of some 991 exposed and carrier stallions and mares in 48 states have failed to confirm the origin of this latest CEM event. Neither clinical evidence of CEM nor decreased pregnancy rates were reported in infected/exposed mares. In light of these findings, was the considerable expense incurred in investigating the latest CEM occurrence warranted? Only time will tell whether the disease has once again been eradicated from the US and if the cost of regaining CEM-free status was justified.

Key Words: CEM, impact, eradication

1097 Use of fluorescent *in situ* hybridization (fish) to identify endometritis pathogens in the mare. M. R. Petersen*, H. Lehn-Jensen, and A. M. Bojesen, *Faculty of Life Sciences*, *Copenhagen, Denmark*.

Presence of bacteria in the uterus as a cause of infertility was suggested more than 80 years ago. Nielsen, JM 2005 demonstrated that the diagnostic sensitivity and specificity by culture was improved when a endometrial biopsy was used compared with a swab. As *Streptococcus equi* ssp. *zooepidemicus* (*S. zoo*) was isolated more often using a biopsy compared with a swab, we decided to determine location of *S. zoo* within the endometrium. FISH can be used to demonstrate the spatial distribution of bacteria within infected tissue. Endometrial biopsies were analyzed from young non-infected research mares and from broodmares from which S. zoo had been isolated. Experimental infections in research mares with S. zoo were carried out and biopsies recovered at specific time points. Using FISH S. zoo could be localized in endometrial biopsies from mares positive for S. zoo. In the research mares large number of bacteria were localized at the luminal epithelia following inoculation, but no bacteria could be visualized at 96 h. In biopsies from chronically infected broodmares the bacterial localization was markedly different. In all of the biopsies from broodmares S. zoo was localized in distinct foci, either below the epithelial lining, within the endometrial crypts or deep in the stratum compactum, but never at the luminal epithelia as seen in the experimentally infected mares. Since this initial study we have performed FISH to visualize S.Zoo in biopsies from broodmares treated with antibiotics. Streptococci are in general sensitive to the antibiotics used for treatment of endometritis, but treatment failure of chronic cases have been described. Eventhough the mares were treated with antibiotics streptococci could still be visualized deep in the endometrium. Since the general perception has been that endometritis is a superficial infection, treatment has not been focused on bacteria localized deeper in the endometrium. We therefore suggest a treatment regimen for chronically infected mares utilizing antibiotics with a capacity to penetrate cell membranes allowing deep tissue penetration.

Key Words: endometritis, FISH, horse

1098 Chronic equine endometritis: What is missed with traditional diagnostics. M. M. LeBlanc*, *Rood and Riddle Equine Hospital, Lexington, KY*.

Endometritis, a major cause of mare infertility arising from failure to remove bacteria, spermatozoa and inflammatory exudate post-breeding, is often undiagnosed. Defects in genital anatomy, myometrial contractions, lymphatic drainage, mucociliary clearance, cervical function, angiosis (damage to arterioles) or inflamm-aging (increased levels of pro-inflammatory cytokines associated with aging) underlie susceptibility to endometritis. Diagnosis is made through detecting uterine fluid, vaginal discharge, abnormal inter-estrous intervals, inflammatory uterine cytology and positive uterine culture. However, these signs may be absent in gram-negative infections. Hypersecretion of an irritating, watery, neutrophilic exudate underlies classic, easy-to-detect streptococcal endometritis. In contrast, biofilm production, tenacious exudate, and focal infection may characterize chronic endometritis, commonly caused by gram-negative organisms, fungi and staphylococci. Clinical signs of chronic endometritis may include dry, splotchy vaginal mucosa, hyperechoic lines visualized ultrasonographically, or absence of intrauterine fluid during estrus only to appear after ovulation. Gram-negative uterine pathogens can be missed on culture swab while cytological specimens may only contain heavy debris and few, if any, neutrophils. Culture of uterine biopsy tissue, or small volume uterine lavage efflux are twice as sensitive as guarded swabs in detecting gram-negative organisms. Uterine biopsy, a technique that has lost favor in equine veterinary practice, may detect deep inflammatory and degenerative changes, such as angiosis, lymphatic lacunae or destructive fibrosis while endoscopy reveals focal lesions invisible on ultrasound. New treatments designed to improve pregnancy rates in mares with endometritis have been evaluated recently. These include lavage one hour before breeding, cloprostenol post breeding, cervical dilators, intrauterine chelators (tris-EDTA), mucolytics (DMSO, kerosene, n-acetyl-cysteine), corticosteroids (predisolone, dexamethasone) and immunomodulators (cell wall extracts of Mycobacterium phlei and Propionibacterium acnes).

Key Words: equine, endometritis, diagnostics