Evaluation of pathogen progression during induced placentitis in mares using *lux*-modified *Escherichia coli* and novel bioluminescence imaging technology

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Placental infection due to opportunistic pathogens such as *Escherichia coli* (*E. coli*) is a common cause of abortion, stillbirth and premature delivery in horses. Moreover, pathogen progression during placentitis may involve invasion of fetal tissues, including the brain, leading to increased pro-inflammatory cytokine expression resulting in onset of premature delivery and/or fetal neurological damage. Thus, the objective of this pilot study was to monitor pattern of pathogen progression and invasion of fetal tissues by experimentally-inducing uterine infection of mares with a *lux* gene-modified *E. coli* using real time bioluminescence (biophotonics) imaging technology. To this end, one horse (~280 d gestation) and two pony (~300 d) mares were inoculated trans-abdominally (ultrasound-guided intra-amnion) with 2 x 10^6 colony forming units of *E. coli* (CFU in 1 mL of broth) transformed with the pAK1-*lux* plasmid (*E. coli*-*lux*). The plasmid (11,904 bp) used is a broad-host-range cloning vector with numerous plasmid replicons. Trans-abdominal and –rectal ultrasonography was performed every 12 h for confirmation of fetal viability. One pony mare and the horse mare aborted ~24 h post-inoculation while the third fetus was recovered at 40 h post-infection following euthanasia of the mare. Fetuses recovered immediately post abortion and the intact uterus of the third mare were subjected to biophotonic imaging using a NightOwl imaging system (Peltier cooled slow scan CCD camera; Berthold Technologies, Oak Ridge, TN, USA) for detection of *lux*-expressing (photon emission) bacteria. Scans were performed over a 5 min period to accumulate photons indicative of pathogen presence in localized tissues. Subsequent to intact uteri and/or whole fetus imaging, fetuses were dissected and heart, lungs, liver, bladder, gastro-intestinal (GI) tract and brain were removed and imaged. *Lux* emitting bacteria were found in the lungs, GI tract, nares and sinuses but not in the brain, heart or liver in the two fetuses recovered at 24 h post-inoculation. Fetal amniotic, GI tract, stomach, bladder, and pericardial fluids were analyzed for presence of emitting bacteria and to determine total bacteria counts. Cultures of amniotic, stomach and bladder fluids confirmed presence of *lux*-emitting bacteria with counts ranging from 10 x 10^6 to 140 x 10^6 CFU/mL for amniotic and GI tract fluid, respectively, but no counts in pericardial fluid. Histopathology confirmed bacterial colonization of the fetal brain at 280 d but not at 300 d, which may suggest differences in stage of cerebral development. No *E. coli*-*lux* emitting bacteria were identified in the fetus or fetal fluids recovered from the mare at 40 h. These data demonstrate that bioluminescence and real time imaging provide a novel means of understanding pathogenesis of bacterial-induced placentitis and preterm birth in horses. The application of this novel imaging technology with *lux*-modified organisms may facilitate the development of more targeted therapeutic interventions.

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