Next generation DNA sequencing, culture and cytology results in 29 mares with suspected endometritis

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Endometritis is a common cause of infertility in the mare, caused most frequently by microbial (bacteria and fungi) colonization of the endometrium. Traditional methods to diagnose infectious endometritis in the mare include aerobic culture and cytologic evaluation of fluid, swabs, or tissue obtained from the uterine lumen or endometrium. Next generation DNA sequencing (NGS) is a method of microbial diagnostics using 16S sequencing that is used in human medicine to identify microorganisms not easily identified by traditional culture techniques. The objective of this study was to compare NGS (PathoGenius Laboratory, Lubbock, TX) results to traditional methods of diagnosing infectious endometritis.

A group of 29 light-horse mares evaluated in clinical practice for reproductive management in central Kentucky were used as subjects. The subjects were 500 -700 kg, mean age 9.4 years and included 25 barren, 1 maiden, and 2 foaling mares. Transrectal palpation and ultrasonography was performed and samples were obtained from subjects with: one ≥ 25mm follicle present on an ovary, moderate uterine edema, and a relaxed cervix. A uterine lavage was performed with 250ml-1L of fluid and the efflux split. One sample was submitted for aerobic culture and cytologic evaluation, the other sample was submitted for NGS. Processing of samples for cytologic evaluation included allowing the fluid to settle for 1h prior to removing 50ml of fluid and centrifuging for 10 minutes at 800g.The pellet was smeared onto a slide and evaluated at 100x for the presence of leukocytes as previously described.[1] Quantitative PCR was performed prior to NGS to determine microbial load. The NGS sequencing was reported as: negative = no microbial DNA isolated, low = less than 10^5 bacteria/fungi/mL, medium =10^5-10^7 bacteria/fungi/mL or high = greater than 10^7 bacteria/fungi/mL. The microbial DNA isolated was listed by genus, species, and the percentage of microbial DNA present.

Aerobic culture of fluids recovered growth on 100% (29/29) samples, 17.2% (5/29) had more than one bacteria isolated, and the most common isolates were: *E. coli* 44.8% (13/29), *beta-hemolytic Streptococcus* 41.4% (12/29), *Pseudomonas sp.* 10.3% (3/29), and *Klebsiella sp*. 6.7% (2/29). Of the samples that had growth following culture, 17.2% (5/29) of these were negative with NGS, 17.2% (5/29) were low, 37.9% (11/29) 2/9) were medium, and 27.6% (8/29) were high. The most common microbe identified on NGS was *E.coli* 37.9% (11/29) with 72.7% (8/11) identified on culture. *Psuedomonas sp* and *Klebsiella sp* were identified in 17.2% (5/29) samples, but only 60% (3/5) and 20% (1/5), respectively, isolated these bacteria with culture. Interestingly only 6.8% (2/9) of NGS samples identified *beta-hemolytic Streptococcus*, and of these only 50% (1/2) were also isolated with culture. Cytologic analysis of 28.5% (8/28) of samples had > 3 leukocytes per hpf, 28.6% (8/28) had 1-2 leukocytes per hpf, and 42.8% (12/28) had no leukocytes observed.

This study describes the NGS results compared to traditional methods for identifying bacterial endometritis in a population of mare's which were identified as having microbial growth isolated from endometrial samples. Further work is required to determine the utility of this diagnostic tool in identifying clinical and subclinical cases of infectious endometritis.

Keywords: Endometritis, equine, next generation sequencing

Reference

1. Riddle WT, LeBlanc MM, Stromberg AJ: Relationships between uterine culture, cytology and pregnancy rates in a Thoroughbred practice. Theriogenology 2007;68:395-402.