Endometritis, a common cause of infertility in the mare, is traditionally diagnosed with 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 describe the Next Generation Sequencing (PathoGenius Laboratory, Lubbock, TX) results compared to traditional methods of diagnosing infectious endometritis in a group of clinically normal mares. A group of 10 clinically normal recipient mares (weight 500-700 kg, mean age 7.3 years, 2 barren, 1 maiden, and 8 mares had foaled > 4 months, and been weaned > 30 days prior to sampling) were used as subjects. Transrectal palpation and ultrasonography was performed and samples obtained from subjects with: one ≥ 30mm follicle present on an ovary, moderate uterine edema, <1 cm anechoic fluid in the dorso-ventral plane, and a moderately relaxed cervix. Endometrial swabs (ES) were obtained through a vaginal speculum using a double-guarded Kalajian swab, one swab was placed in Amies medium and submitted for aerobic culture, the other swab in a red top tube was submitted for NGS. Endometrial cytology using the cap from a double-guarded swab was obtained and submitted for cytologic analysis as previously described.1 Small-volume lavage (SVL) of the uterine lumen was performed using 1 L of sterile lactated Ringer’s solution, and the efflux was split into two 50 mL conical tubes, 1 submitted for aerobic culture and cytology, the other submitted for NGS. An endometrial biopsy was obtained and swabbed and submitted for aerobic culture and NGS. Quantitative PCR was performed prior to NGS to indicate microbial load, NGS sequencing was reported as: negative = no microbial DNA isolated, low = less than 10^5 bacteria/fungi/mL, or greater than 10^5 bacteria/fungi/mL and the microbial DNA isolated listed by genus, species, and the percentage of microbial DNA present. Aerobic culture of ES recovered no growth on 90% (9/10) samples, and 77.8% (7/9) of these were negative with NGS and 22.2% (2/9) were low, (E.ictaluri and S.epidermis were the primary microbes identified). Aerobic culture of 10% (1/10) of ES recovered a Bacillus species with a low NGS result (Corynebacterium the majority of DNA identified). Aerobic culture of SVL recovered no growth on 70% (7/10) of samples, and 30% recovered growth of one organism (E.coli, α-streptococcus, and β-streptococcus). Next Generation DNA sequencing analysis of SVL recovered negative results on 70% (7/10), and low results on 30% (3/10) with E.coli, P.aeruginosa, C.xylanorovans isolated, none of which grew on aerobic culture. Swab of endometrial biopsies recovered no growth on aerobic culture of all samples, and only one sample NGS detected low levels of B.simplex. Cytology results from endometrial swabs were 80% (8/10) normal, and 20% (2/10) had moderate inflammation. Cytology results from SVL recovered no neutrophils on 60% (6/10) of samples and 1-2 leukocytes/100x magnification on 40% (4/10) of samples. The present preliminary study describes the NGS results compared to traditional methods for confirming bacterial endometritis in a normal population of mares. Further controlled studies are required to determine the effectiveness and utility of this diagnostic tool in identifying clinical and subclinical cases of infectious endometritis.

Keywords: Endometritis, equine, Next Generation Sequencing

Reference