ABSTRACTS

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Canine pyometra - A novel in vitro model

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INTRODUCTION: Pyometra is a common uterine disease of intact middle-aged bitches and is most commonly associated with Escherichia coli (1). Its pathogenesis is incompletely understood. In a disease model we have shown that dogs are particularly susceptible to inoculation of E. coli during diestrus, when uterine tissues are under the influence of progesterone. Inoculation in estrus or anestrus did not result in pyometra (2).

OBJECTIVES AND METHODS: In order to investigate the bacteria-host interactions leading to pyometra it is necessary to i) manipulate gene expression in pathogenic E. coli; ii) reliably simulate different stages of the canine estrous cycle; and iii) assess the interactions between E. coli and the canine immune response. We have developed a model that addresses all of these requirements.

Genes in E. coli isolates from canine pyometra cases were insertionally inactivated with a selective marker. E. coli strains were further transformed with an expression vector encoding green fluorescent protein (GFP).

Ovariectomized greyhound bitches were treated with oestradiol benzoate (Intervet, Bendigo East, Victoria, Australia) at a daily dose of 0.6 to 4.8 μg/kg, intramuscularly, for 13 days. This was followed by 2 mg/kg megestrol acetate (Jurox Pty Ltd., Rutherford NSW, Australia), orally, once a day for 3 days or for 16 days to simulate estrus or diestrus, respectively. Uteri were obtained either on day 4 of simulated estrus or on day 10 of simulated diestrus. Untreated animals served as anestrous controls.

Uteri were obtained either on day 4 of simulated estrus or on day 10 of simulated diestrus. Untreated animals served as anestrous controls. Endometrial biopsies were incubated in vitro with E. coli for up to 5 hours and qPCR assays were used to assess β-defensin mRNA levels. To expand the scope of cycle-specific gene expression patterns, RNAs from 4 dogs per group (diestrus, estrus and anestrus) were pooled to produce a cDNA library that was then analyzed using Illumina deep-sequencing.

RESULTS: Bacterial incubation with endometrial tissue allowed quantification of bacteria adherent to the endometrium in competitive binding studies and morphological assessment of this binding using fluorescent microscopy. Results thus far have shown that some E. coli isolated from cases of pyometra can compensate for the loss of all of the known adhesins (FimH, PapGIII and Sfa). However, E. coli expressing a single adhesin (FimH) lost their capacity to bind if this adhesin was absent.

Preliminary analysis of the RNA-Seq data revealed profound cycle-dependent expression differences in a number of genes involved in innate immunity. We have found that pooled RNA-Seq data correlate well with individual qPCR assays and that these data may be used as a valuable tool for generating hypotheses about the pathogenesis of pyometra. Using RNA-Seq and quantitative PCR we demonstrated a 100-fold increase of the antimicrobial peptides canine β-defensin 1 and β-defensin 139 mRNA expression in the canine endometrium during diestrus, compared to anestrus and estrus.

CONCLUSION: This novel in vitro bacteria-host interaction model allows characterization of bacterial factors and early infection response pathways under controlled conditions allowing estrus cycle stage-specific changes in gene expression patterns to be assessed. By combining the specificity of qPCR with the range of profiles generated by high-throughput sequencing, we are now able to more fully characterize cycle-dependent factors that modulate intra-uterine responses to E. coli.