ABSTRACTS

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Effect of simulated stages of the canine estrous cycle on Escherichia coli binding to canine endometrium

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INTRODUCTION: Pyometra, a prevalent infectious uterine disease that affects intact middle-aged bitches, is typically associated with E. coli. We have shown in a disease model that dogs are particularly susceptible to E. coli infection during diestrus, when uterine tissues are under the influence of progesterone (1). Inoculation in estrus or anestrus does not result in pyometra. Intra-uterine microbial persistence and subsequent infection is preceded by attachment of E. coli to mucosal membranes via uropathogenic virulence factors (2). This bacterial binding might differ during different stages of the canine estrous cycle (3, 4).

OBJECTIVES AND METHODS: Our hypotheses were i) that bacterial adhesion to canine endometrium differs between different stages of the estrous cycle and ii) that the adhesin FimH facilitates this adhesion.

Twelve post-pubertal, ovariectomized greyhound bitches were treated with estradiol 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 (n=4 per group). Untreated animals served as anestrous controls (n=4). This experiment was conducted with the approval (# 0811068.1) of the Animal Experimentation Ethics Committee, Faculty of Veterinary Science, The University of Melbourne.

Tissue samples from each uterus were washed separately in phosphate buffered saline and incubated with a pathogenic E. coli strain carrying the fimH gene, but no other adhesin genes (P4-wt) or an E. coli strain in which fimH was insertionally inactivated (P4-ΔfimH::Kan), or with phosphate buffered saline as a negative control. Transformed bacteria also carried a GFP reporter plasmid. After washing, tissue samples were either frozen in liquid nitrogen or homogenized and inoculated onto nutrient agar for quantification of adherent bacteria. Cryostat samples were examined for the presence of the two bacterial strains.

RESULTS: The concentration of viable P4-wt bound to the tissue samples was $10^{5.71\pm0.95}$, $10^{5.05\pm0.76}$ and $10^{5.09\pm0.54}$ colony forming units/cm² in simulated diestrus, estrus and anestrus, respectively. The mean number of P4-ΔfimH::kan bound was $10^{3.54\pm0.79}$, $10^{2.85\pm0.88}$ and $10^{3.20\pm1.23}$ in simulated diestrus, estrus and anestrus, respectively.

The differences in binding to canine endometrium at different stages of the estrous cycle were not significant. However, the mean difference in binding of the P4-wt and the P4-ΔfimH::kan across all stages of the simulated estrous cycle was $10^{2.08\pm0.55}$ and was significant (P < 0.001 by paired t-test on geometric means).

CONCLUSION: In summary, we were unable to identify any significant correlation between the stage of the cycle on binding of the pathogenic E. coli strain P4, but did show that disruption of the fimH gene in the P4 strain significantly reduces binding to canine endometrium in vitro across all stages of the cycle. Individual differences in numbers of P4-wt bacteria bound between dogs might suggest differences in genetic or epigenetic differences in FimH receptor expression by the endometrium, unrelated to the stage of the estrous cycle.


