The role of strain-specific adhesin genes in binding of pyometra-inducing *E. coli* to canine endometrium

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*Escherichia coli* (*E. coli*) is the most commonly isolated infectious agent causing pyometra in bitches. The adhesin *FimH* has been shown to facilitate bacterial attachment to canine endometrium. However, many *E. coli* strains isolated from the uteri of infected dogs carry several adhesin genes (*fimH*, *papGIII* and *sfa*). The objective of this study was to investigate the role of each adhesion gene product, acting alone or expressed in combination, in the bacterial binding to canine endometrium.

*E. coli* strain P3, which was isolated from a uterus of a bitch naturally affected with pyometra, was shown by PCR to carry all three known adhesin genes. Knockout (KO) mutants of this wildtype (P3-wt) strain were generated using insertional inactivation. Single (P3-ΔfimH::Kan; P3-Δpap::Cm; P3-Δsfa::Kan), double (P3-ΔfimH::Kan-Δpap::Cm; P3-ΔfimH::Kan-Δsfa::Kan; P3-Δpap::Cm-Δsfa::Kan), and triple (P3-ΔfimH::Kan-Δpap::Cm-Δsfa::Kan) mutants were produced. Adhesion assays on anoestrous uteri of three post-pubertal bitches were undertaken. Full-thickness tissue samples were collected using a 6 mm biopsy punch. Tissue samples from each uterus were washed separately in PBS and incubated with the P3-wt or P3-KO strains, or with PBS as a negative control. After washing, tissue samples were homogenized and plated on nutrient agar for determination of colony forming units (CFU)/cm² of tissue.

Overall, the number of bacteria adhering to canine endometrial biopsies were comparable and no significant difference in the number of bound bacteria was found between the P3-wt strain and the single or double KO-strains. However, the triple knockout strain (P3-ΔfimH::Kan-Δpap::Cm-Δsfa::Kan) displayed less binding to the canine endometrium compared with the P3-wt strain (p=0.034; by Dunnett’s simultaneous test).

This study shows that a pathogenic *E. coli* strain (P3) isolated from the uterus of a bitch with pyometra was able to fully compensate for the loss of two of its three known adhesin genes. It was necessary to inactivate all three known adhesin genes in order to see a significant decrease in binding to canine endometrium. However, the triple knockout mutant still retained 42% binding capacity compared with the P3-wt strain.

This retained binding contrasts with similar analyses of a different *E. coli* strain, P4, in which functional loss of the single adhesin gene (*fimH*) eliminated >99% of bacterial binding capability. These combined studies suggest that pathogenicity varies between *E. coli* strains. Therefore, future research should focus on the interaction of bacterial virulence and host immunity in order to further elucidate the pathogenesis of canine pyometra.

**Keywords:** Adhesin, dog, *E. coli*, pyometra

**Reference**