Uropathogenic virulence factor FimH facilitates binding of pyometra-causing *E. coli* to canine endometrium

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Pyometra is a prevalent uterine infection that affects intact middle-aged bitches and *Escherichia coli* is the most common isolate. The adhesin FimH is an important virulence factor which contributes to colonization of the urinary tract by uropathogenic *E. coli*.

The objective of this study was to demonstrate that FimH also facilitates binding of *E. coli* to canine endometrium.

Our hypothesis was that disruption of *fimH* expression would lead to a reduction in bacterial binding to uterine epithelial cells. An *E. coli* strain (P4), isolated from a clinical case of canine pyometra, was demonstrated by polymerase chain reaction to carry the gene encoding FimH but no other known *E. coli* adhesins. The chromosomal gene *fimH* was insertionally inactivated with an antibiotic resistance cassette to generate a knock-out mutant (∆*fimH::Kan*). The P4 wildtype strain (wt) and ∆*fimH::Kan* were further transformed with an expression vector encoding for a green fluorescent protein (GFP; Clontech Laboratories, Palo Alto, CA, USA).

Adhesion assays were used to compare the binding of the wt and ∆*fimH::Kan* to canine endometrium *in vitro*. Anestrus uteri from five bitches were obtained from routine hysterectomies and full-thickness samples were collected using a 6 mm biopsy punch. Tissue samples from each uterus were washed separately in PBS and incubated with P4 wt or ∆*fimH::Kan*, or with PBS as a negative control. After washing, tissue samples were either frozen in liquid nitrogen or homogenized and plated on nutrient agar for determination of colony forming units (CFU)/g of tissue. Thin sections of frozen samples were evaluated for the presence of green fluorescent bacteria.

Adhesion of both bacterial strains to the endometrium was observed by fluorescent microscopy but ∆*fimH::Kan* was considerably less adherent than the wt. This finding was confirmed by viable bacterial cell counts as determined by CFU/g tissue. Binding of ∆*fimH::Kan* was only 3% of that of the wt. The mean difference in binding between the two strains on the log10 scale was 2.5 (SD 0.37) (p < 0.001 as per paired t-test). Complementing the mutation in ∆*fimH::Kan* restored the phenotype of the wt binding.

In summary, we demonstrated that disruption of the *fimH* gene in the pathogenic *E. coli* P4 strain significantly reduced bacterial binding to canine endometrium *in vitro*. Future studies targeting uropathogenic virulence factors to prevent binding of *E. coli* to the endometrium might reduce the incidence of pyometra in dogs.

**Keywords:** Dog, *E. coli*, FimH, uropathogenic virulence factors, pyometra