Evaluation of biofilm production by *Escherichia coli* isolated from clinical cases of canine pyometra

T.E. Fiamengo, a E.E. Runcan, a C. Premanandan, b M.A. Coutinho-da Silva a

aDepartment of Veterinary Clinical Sciences; bDepartment of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH

Many strains of *Escherichia coli* (*E. coli*) have the ability to produce biofilm, a matrix of extracellular polymeric substances that confers antimicrobial resistance, leading to increased morbidity and recurrent infections. In dogs, production of biofilm by *E. coli* has been observed in bacteria isolated from recurrent cases of urinary tract infections. However, to date, studies addressing biofilm presence during canine uterine infections caused by *E. coli* are lacking. The objective of this study was to determine the role of biofilm production by *E. coli* during uterine infection by: 1) confirming the presence of biofilm *in situ*, and 2) determining the ability of different strains of *E. coli* to produce biofilm *in vitro*. We hypothesized that most strains of *E. coli* involved in canine pyometra will be capable of producing biofilm both *in vivo* and *in vitro*. Samples used in this study were obtained during ovariohysterectomy of dogs affected by pyometra (n=13). A swab of the uterine contents was collected immediately after surgery and submitted for aerobic culture. Two sections of the uterine horns were preserved in 3% glutaraldehyde and 10% buffered formalin. At approximately 24 h of culture, isolated bacteria were identified by matrix assisted laser desorption/ionization and *E. coli* isolates were frozen at -80˚C. Only tissue samples from cases confirmed to have *E. coli* infection were evaluated by scanning electron microscopy (SEM), histopathology and biofilm assay. During SEM, the surface of the endometrium was evaluated for the presence of bacteria and/or a fibrous matrix. Sections submitted for histopathology were stained with hematoxylin and eosin and periodic acid-Schiff (PAS) and evaluated by a board certified veterinary pathologist blinded to treatments. *E. coli* isolates were analyzed for biofilm formation by microtiter biofilm assay using crystal violet. Optical densities were compared by analysis of variance (ANOVA), using StatPlus software. Significance was set at P<0.05. Nine out of thirteen cases (69%) resulted in pure growth of one or two *E. coli* colonies, totaling 11 different isolates. Areas suggestive of the presence of biofilm were observed on all samples on SEM; however, bacteria consistent with *E. coli* were only visualized in three samples. All tissues exhibited endometrial inflammation with varying degrees of luminal exudate on histopathology. Visible bacteria were observed in five specimens. Mucus was located within cystic endometrial glands and occasionally overlying epithelium in seven specimens. Nine isolates (9/11, 82%) had significantly higher optical densities than negative controls, indicating *in vitro* biofilm production. In conclusion, we demonstrated that clinically relevant strains of *E. coli* produce biofilm both *in vivo* and *in vitro*, supporting our hypothesis. Development of new treatment modalities for pyometra aimed at disrupting biofilm may enhance therapeutic efficacy allowing for preservation of the reproductive potential of genetically valuable dogs.

**Keywords:** *Escherichia coli*, biofilm, canine, pyometra