Endometritis in dogs – current knowledge and future considerations

S.K. Lyle

Department of Veterinary Clinical Sciences School of Veterinary Medicine,

Louisiana State University, Baton Rouge, LA

Abstract

Canine endometritis, as an entity separate from cystic endometrial hyperplasia, is a poorly understood process, yet may be responsible for infertility. Inflammatory infiltrates of the endometrium in the absence of proliferative changes are the hallmark of this disease. The ascension of bacteria from the vagina through the cervix is theorized to lead to a chronic, low-grade endometritis, which does not produce overt clinical disease, such as that seen with pyometra. *Escherichia coli* is the organism most commonly implicated in pyometra, and is likely the most common organism involved with endometritis. Various uropathogenic virulence factors (UVFs) have been identified from uterine isolates, many of which share common UVFs from isolates producing urinary tract disease in dogs and humans. Of these, P fimbria is thought to be crucial for initial bacterial adherence to the luminal epithelium. Reduction in Mucin-1 expression and immunolocalization in endometrial epithelial cells in early diestrus may be involved with producing a permissive state for bacterial adhesion, allowing colonization. Samples that may aid in the diagnosis of endometritis include vaginal or uterine cultures, uterine biopsy, and possibly endometrial cytology and ultrasonography. Treatment of endometritis is focused on elimination of infection through the use of antimicrobial agents, and physical clearance of the uterus through the use of prostaglandins, dopamine agonists, and progesterone-receptor antagonists.

Keywords: Dog, infertility, endometritis, diagnosis, treatment

Introduction

Although many clinicians might empirically agree that inflammation of the endometrium may contribute to a reduction in fertility, direct evidence of a causal effect of endometritis on reducing fertility is lacking. Subfertility and infertility due to cystic endometrial hyperplasia is more generally agreed upon; however, whether the reduction in fertility is due to the proliferative response of the endometrium or the inflammatory component of the process is unknown.

Histopathology of the canine endometrium

Endometrial hyperplasia, with or without cystic changes is considered a post-estrual luteal phase disease. Age and nulliparity are considered risk factors for development of CEH.[1,2] Two main hypotheses exist on the pathogenesis of CEH and subsequent development of pyometra. The classical description involves the development of endometrial hyperplasia which subsequently leads to an inflammatory reaction. The accumulation of secretions from the hyperplastic endometrium supports the growth and proliferation of bacteria which ascend through the cervix. Establishment of bacterial infection leads to the accumulation of exudate (pyometra). An alternative hypothesis centers on the premise that chronic low-grade uterine infection drives the endometrial proliferative response either by bacterial toxins or inflammatory mediators.[3] It has also been suggested that endometritis-pyometra can occur independent of endometrial hyperplasia; unfortunately the age distribution of these cases were not defined.[4] A variety of stimuli have been used to experimentally reproduce endometrial

proliferation. These have ranged from china balls,[5] suture,[6-8] and bacteria,[9-13] among others. The proliferative changes observed have been well-characterized, and are not the focus of this discussion. For detailed information on CEH, the reader is directed to several excellent reviews on the topic.[1,3,4,14]

Endometritis is also a common finding, and was recently determined to be the most common diagnosis (94 cases) in a survey of 366 canine endometrial biopsies. Hyperplasia was the second most common diagnosis (86 cases).[15] It is most common to find a plasmacytic infiltrate with subclinical endometritis[14] or with CEH with plasmacytic infiltration and little intraluminal fluid accumulation,[1] progressing to neutrophilic plasmacytic infiltrates in cases of CEH and significant fluid accumulation (pyometra).[1]

Microflora of the reproductive tract

Debate exists on whether vaginal cultures are useful for determining the presence of intrauterine infection. Although most prepuberal and postpuberal bitches were found to have positive vaginal cultures, the majority of uterine cultures were found to be negative.[16] Similarly, all uterine swabs collected from late diestrus, progestin-supplemented bitches were sterile,[17] although concurrent sampling of the vagina was not performed. The predominate isolate from infected uteri is *Escherichia coli*,[1,18-22] with reported incidences of 73%,[1] 79.4%,[21] and 85%.[22] Although *E. coli* in pure culture was the most common isolate from bitches with pyometra, mixed cultures were the most common finding in 'infertile' bitches, leading to the conclusion that vaginal cultures have low diagnostic value.[20] Conversely, other studies found the uterus not to be a sterile environment,[23,24] and that vaginal isolates reflected those of the uterus.[23] Bacteria were

consistently recovered from the uterus during proestrus and estrus, and post-mortem uterine isolates always reflected those of the cervix and vagina.[24] The most common uterine isolates were *E. coli*, *Haemophilus* spp., α-hemolytic streptococci, *Corynebacterium* spp., *Streptococcus canis*, *Alcaligenes faecalis*, *Bacteroides* spp., *Pasteurella* spp., and *Proteus mirabilis.*[24] A method to transcervically collect uterine secretions that is guarded from vaginal secretions would enhance the ability of clinicians to accurately diagnose the presence of bacteria in the uterus.

Several authors have investigated virulence factors of *E. coli* isolates from cases of pyometra. Early characterizations focused on the presence of the O-[18] and K-antigen[21,22] Uropathogenic *E. coli* strains, which are responsible for urinary tract infections in dogs and cats, may originate from the intestinal tract, and possess a cluster of virulence-related genes encoding for specific O-antigens, type 1 fimbriae, P fimbriae, S fimbriae, α-hemolysin, cytotoxic necrotizing factor 1, and aerobactin (iron-sequestering system).[25-27] These strains are not canine-specific, and it has been suggested that the dog may serve as a source of uropathogenic *E. coli* for human urinary tract infections (UTI).[28,29] Biochemical fingerprinting of *E. coli* isolates from pyometra and UTI suggest that these isolates originate from the fecal flora, and the same clone of *E. coli* is present in cases with concurrent UTI and pyometra.30 Similarly, DNA-profiles of *E. coli* isolates from the urinary bladder and uterus of bitches affected simultaneously with UTI and pyometra were 100% identical, and that all colonies from a site were identical, despite macroscopic morphologic differences.[31] The *papGIII* allele, the most frequent allele encoding for P fimbriae in canine and human uropathogenic *E. coli* isolates,[29] had a significantly higher prevalence in *E. coli* isolates

from pyometra, and the proportion of strains from pyometra possessing more than three uropathogenic factors was greater than that of fecal strains.[32] The presence of P fimbriae is thought to be crucial for bacterial adherence to epithelial cells of the urinary tract. The PapGIII adhesion binds to Galα1-4 Galβ-containing glycolipid receptor and its coreceptor TLR4 present on urinary epithelial cells.[33] The presence of these receptors has been confirmed for canine urinary epithelium,[34] but has not been investigated in canine endometrium. Other virulence genes that have been associated with uropathogenicity and were present in high proportions of isolates from pyometra isolates include *fim* (Type I fimbriae) and *sfa* (S fimbriae), although the differences were not statistically significant.[32] Although Type I fimbriae are present on many isolates from human UTIs, the correlation with pathogenicity is considered low.[35] *Escherichia coli* bearing S fimbriae bind to human renal proximal tubular cells,[36] but only 27.4% of strains were positive for *sfa* compared to 97.5% of strains carrying *fimH* (Type I fimbriae).[37]

These findings have led researchers to the conclusion that the pathogenesis of both UTI and pyometra involve ascension of intestinal strains of *E. coli* into the lower urinary tract, cranial vagina, and uterus.[28,30,32] Only a few investigators have attempted to induce infection by inoculation with *E. coli*. In a series of investigations using an *E. coli* isolate from a clinical case of pyometra, Nomura et al. inoculated the uterus of dogs in either pro-estrus/estrus, diestrus, post-partum, or anestrus with or without cervical ligation.[9,11,12] When examined 12 d post-inoculation with cervical ligation, the incidence of pyometra in proestrus/estrus, diestrus, post-partum, and anestrus was 100%, 100%, 80%, and 28%, respectively.[9] When examined 12 d post-inoculation without cervical

ligation, the incidence of pyometra in pro-estrus/estrus, diestrus, post-partum, and anestrus was 25%, 89.9%, 70.6%, and 50.6%, respectively. More recently, 5×10^7 CFU of an *E. coli* (O2:H:K) isolated from a clinical case of pyometra was inoculated in the uterus of intact bitches at either post-LH day 1-10, 11-20, 21,30, 31-40, 41-50, or 51-60; the incidence of pyometra induced was 16.7%, 90.9%, 78.9%, 62.5%, 40.0%, and 0%, respectively. Bitches with induced pyometra were either treated with dinoprost tromethamine and enrofloxacin, or were allowed to spontaneously recover. There was no difference in pregnancy rates between treated and non-treated bitches on the subsequent estrus, and recurrence of pyometra did not occur.[13] While this model did induce pyometra in the strict sense of the definition (the presence of pus in the uterus during the luteal phase), the subsequent fertility and lack of recurrence do not fit the typical clinical scenario of bitches with spontaneous pyometra. This model appears to more closely approximate endometritis than pyometra. Subsequently, inoculation of the uterus with an E. coli strain possessing five UVFs induced CEH/pyometra in diestrus-simulated ovariectomized bitches, while inoculation into the vagina failed to establish uterine infection or endometrial changes.[10] Differences in these two studies lie in the status of the bitch and potential difference in the pathogenicity of the *E. coli* strain.

Host-pathogen interactions

Limited investigation on the host-response to intrauterine infections exists, but a few noteworthy studies shed some light on mechanisms by which bacterial are able to colonize the endometrium. The proliferative response of peripheral blood monocytes (PBMCs) to a clone of *E. coli* isolated from the uterus from a dog with pyometra was significantly decreased at day 10 of diestrus compared to proestrus, estrus, day 30 of

diestrus, or anestrus.[38] Similarly, the addition of progesterone or 5α-dihydroprogesterone to PBMCs collected from anestrous bitches significantly reduced the response to *E. coli* compared to PBMCs supplemented with estradiol 17-β, 17α-hydroxyprogesterone, or pregnenolone; and progesterone reduced the expression of IFNγ by PBMCs compared to estradiol.[38]

Recently lactoferrin, an antimicrobial and immunomodulator member of the transferrin gene family which is expressed by epithelial cells and neutrophil granules, has been identified in the equine and canine endometrium.[39,40] Lactoferrin's antibacterial property lies within its ability to sequester free iron, thereby inhibiting bacterial growth. In the mare, lactoferrin expression was upregulated during early estrus, protein staining was uninfluenced by cycle and was most intense in the glandular epithelium, and expression of lactoferrin was only increased in mares with delayed physical clearance during early estrus,[39] which might represent a response to inflammation. The pattern of lactoferrin expression has also been described in the bitch, where expression increased from proestrus to estrus, then significantly decreased from estrus to day 10 of diestrus, remaining low at day 35 of diestrus and anestrus; a similar pattern was observed with immunohistochemical staining for lactoferrin,[40] leading the investigators to conclude that estrogen was involved with the regulation of lactoferrin expression. Although reduced lactoferrin expression during diestrus would be a plausible explanation of reduced microbial defenses and increased susceptibility to infection, lactoferrin expression was increased in bitches with pyometra,[40] similar to what was observed in mares with delayed uterine clearance and post-mating induced endometritis.[39] Increased lactoferrin expression in both instances may due to an influx

of neutrophils.[41] While intriguing from a perspective of host-pathogen interactions, unless diminished response in lactoferrin expression and production were observed in bitches suffering from pyometra, reduced lactoferrin activity is unlikely to be responsible for increased susceptibility to infection.

Mucin-1 (Muc1) is an important component of the epithelial cell glycocalyx, functioning as an anti-adhesive molecule; loss of Muc1 expression is considered an integral step in allowing adhesion between the trophoblast and the luminal epithelium.[42] In normal, cyclic bitches, Muc1 expression and localization was significantly decreased at day 10 of diestrus and in bitches with pyometra compared to proestrus, estrus, day 35, or anestrus. Additionally, Muc1 expression and adherence of E. coli to endometrium was inversely correlated.[43] Clearly, further research is needed in the area of host-pathogen interactions of the canine uterus.

Diagnosis of endometritis

If bacteria such as *E. coli* can serve as a stimulus for endometrial proliferation, and hence cystic endometrial hyperplasia; then early diagnosis and appropriate therapy might lead to prolongation of the fertile lifespan of some bitches. For additional detail on specifics for collecting reproductive tract tissues, the reader is directed to an excellent previous review on this topic.[44]

Cultures – The most common method used clinically is guarded culture of the anterior vagina during proestrus. As discussed previously, these may or may not reflect a potential uterine pathogen, but in cases in which significant intrauterine infection is suspected, it is the opinion of this author and others,[44] that this method should yield a satisfactory sample. It is also possible to collect intrauterine secretions following

transcervical catheterization with a 4 to 7 Fr catheter through a rigid cystoscope or endoscope in the standing bitch.[45] Unfortunately this sample would also suffer from contamination by vaginal fluids. Hysteroscopy in the anesthetized bitch may lessen the contamination, but caused petechia or ecchymoses in 50% of cases, and poor visualization in 37.5% of cases.[45] The method providing the most accurate sample of uterine secretions would be that obtained during hysterotomy. In most instances this sample would be obtained concurrent to uterine biopsy.[46]

Cytology – Endometrial cytology is a commonly used diagnostic tool for the diagnosis of equine endometritis; however, it is not routinely used for evaluation of canine cases. Watts et al.[47] described the endometrial cytology of the normal bitch in samples collected by transcervical catheterization or at post-mortem.[45] Endometrial cells were present at all stages, and exhibited degenerative changes during late diestrus, anestrus, and postpartum. During proestrus and estrus, healthy endometrial cells, neutrophils and bacteria were commonly observed. During diestrus and early pregnancy, healthy endometrial cells and neutrophils were most common. During late diestrus and anestrus, evidence of endometrial cell degeneration was observed and lymphocytes and macrophages were the most common leukocyte present. To the authors knowledge there are no published reports regarding changes in endometrial cytology with uterine pathology such as endometritis or cystic endometrial hyperplasia.

Uterine biopsy – Biopsy of the endometrium is maximally invasive, yet provides the most accurate sample for the diagnosis and prognosis for endometritis or other uterine pathology. Samples can be obtained by either laparotomy,[46] or by a transcervical

approach;[48] however, the later technique only provided a diagnostic sample 31% of the time and was associated with hematomucometra in 44% of cases.

Ultrasonography – Ultrasonography is commonly used to diagnose cystic endometrial hyperplasia with or without pyometra. Its usefulness for the diagnosis of endometritis has not been correlated with other diagnostic techniques.

Treatment of endometritis

From the preceding discussion, it is apparent that arriving at an accurate diagnosis of endometritis may be difficult. No controlled studies have been done on therapeutic regimes for the treatment of endometritis. Therefore, treatment options are based on what has been recommended for medical management of pyometra, which is focused on eliminating bacterial infection, if present, and stimulating physical clearance of the uterus. The former is achieved by appropriate antimicrobial agents, the latter by terminating the luteal phase through the use of prostaglandins, dopamine agonists, or progesterone-receptor antagonists, and by stimulation of myometrial contractions through the use of prostaglandins. Prior to initiating medical therapy, detailed owner counseling regarding the intended use and breeding value of the bitch should occur. Ovariohysterectomy should be recommended for bitches without significant reproductive value.

Antimicrobial agents – When at all possible, antimicrobial agents should be chosen based on results of culture identification and sensitivity patterns. The injudicious use of antibiotics has the potential to select for strains of bacteria with greater antibiotic resistance patterns. To the author's knowledge multi-resistant *Staphylococcus aureus* (MRSA) has not been isolated from a canine case of endometritis or pyometra. This

would be a dire situation indeed. Recently, MRSA has been recovered from the uterus of mares with extensive history of intrauterine antibiotic therapy.[49] Systemic administration in the bitch is the most commonly used route of delivery. Although transcervical delivery of antibiotics into the lumen is possible, it is doubtful that uniform distribution of drug to the entire endometrial surface would occur. Additionally, daily treatment would be required; repeated catheterization adding considerably to the cost of treatment. Systemic administration allows for longer treatment regimes (10-14 d), with good penetration of the endometrium.

Prostaglandin – Prostaglandin has been used for many years for the treatment of pyometra.[50,51] Typical doses of $PGF_{2\alpha}$ (dinoprost tromethamine) range from 100 to 500 µg/kg, SQ, one to three times daily, and are considered luteolytic. Lower doses (10 to 50 µg/kg, up to three to five times daily) can also be used to achieve uterine evacuation.[52] The use of prostaglandin in the dog is extra-label; therefore, informed client consent is recommended. Transient (20 to 30 min) side effects of salivation, defecation, urination, and emesis are not uncommon at the higher doses, but are drastically reduced when lower doses are used.

Dopamine agonists – Inhibitors of prolactin will aid in the rapid reduction in progesterone concentrations, and are frequently combined with low-dose prostaglandin protocols for the treatment of induced abortion.[53,54] Bromocriptine (25 µg/kg, PO, q8 to 12 h) or cabergoline (5 µg/kg, PO, q24 h), in combination with prostaglandins, can lead to luteolysis with 24 h.

Progesterone-receptor antagonists – Although not available in the United States, aglepristone has been used for pregnancy termination,[55] and for treatment of pyometra

alone[56] or combination with cloprostenol for pyometra.[57] In a recent study the overall success rate for treatment of pyometra (35 open, 17 closed) with cloprostenol (1 µg/kg) and agelpristone (10 mg/kg, daily) was 84.4% compared to 60% for aglepristone alone; the recurrence rate at 12 and 24 mo was 13% and 19% respectively.[58]

Discussion

It would seem that there is rather compelling evidence to suggest that subclinical endometritis may precede the development of clinically evident CEH and pyometra. The difficulty lies in the ability to render a diagnosis prior to development of proliferative changes in the endometrium. Clearly further research is needed to prove a causal relationship between these two entities. Comparison of *E. coli* strains possessing or lacking UVFs in a model would be a useful first step in that process. Further investigation of host-defense mechanisms, such as the presence of toll-like receptors on the endometrium, cytokine signaling involved with endometritis and proliferative changes of the endometrium would also be informative. Biofilms have been described in a variety of mucosal systems, and provide a mechanism for pathogen evasion of host recognition and protection from certain treatment modalities. Uropathogenic E. coli producing biofilms are implicated in chronic urinary tract infections in people,[59] and it has been suggested that some cases of E. coli endometritis produce biofilm.[60] From a diagnostic standpoint, a guarded system for transcervical collection of uterine secretions in the standing bitch would benefit not only research endeavors, but collection of diagnostic samples from clinical cases. Such information is needed to render an accurate diagnosis, and to progress towards effective treatment strategies.

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Table 1
Function, classification, and distribution of virulence factors of *Escherichia coli* isolates from various species.

Factor	Serogroup or Genes	Site	Species
O Antigens thermostabile; agglutinating; immunogenic O-specific polysaccharide of the cell wall lipopolysaccharide; possessed by all smooth forms of *E. coli*	O1, O2, O4, O6, O25	urinary tract [28]	dog [26,28] human [28]
	O1, O2, O4, O6, O7, O8, O22, O23, O25, O32, O45, O75, O83, O88 O147	uterus [18,21]	dog [18,21]
type I fimbriae present on most E. coli; bind cell-bound and secreted mannosylated glycoproteins, Tamm Horsfall protein, and uroplakins of bladder epitheliam; presumed to be able to bind to endometrium	*fim (pil)*	urinary bladder [33] feces [25, 32] uterus [32]	dog [25, 32] mouse [33]
P fimbriae mediate attachment to Galα1→4Galβ-containing glycolipid receptor and coreceptor TLR4	*papGIII*	urinary epithelial cells [25,28,33] feces [25,32] uterus [32]	human [28] dog [25,28,32]
S fimbriae bind eukaryotic glycoproteins with a terminal α-sialic acid; bind laminin and plasminogen; may play a role in penetration of *E. coli* across basement membrane	*sfa*	urinary tract [25,28]] uterus[25,32] feces [25,32]	dog [25,28,32] humans [25,28]
α-hemolysin common exotoxin; toxic to a wide variety of mammalian cells	*hlyA*	urinary tract [25,28] uterus [32] feces [25,32	dog [25,28,32] human [25,28]
Cytotoxic Necrotizing Factor 1 belongs to a group of bacterial necrotic substances; associated with outer membrane vesicles; activates Rho GTPases of host cell leading to macropinocytosis by epithelial cells; may function as a means of entry and survival in epithelial cells	*cnf1*	urinary tract [25,28] uterus [32] feces [25,32]	dog [25,28,32] human [25,28]
Aerobactin and other iron-sequestering systems bacterial siderophores (low molecular weight Fe(III)-chelator)	*iuc or aer, fyuA, iutA, iroN*	urinary tract [25,28] uterus [32] feces [25,32]	dog [25,28,32] human [2528]