Reproductive microbiome alterations: canine pyometra

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Abstract

Pyometra is a life-threatening uterine infection that affects 20 - 25% of reproductively intact bitches. Canine pyometra is characterized by accumulation of leukocytes and exudate within the uterine lumen. Although exact temporal and progressive mechanisms underlying its pathogenesis are not fully understood, a combination of endocrine, structural, inflammatory and bacterial factors are likely involved in most cases. Pyometra develops during luteal phase; progesterone has a key role in establishment of infection with opportunistic bacteria. Cystic endometrial hyperplasia results from proliferation of endometrial glands and is a predisposing factor for development of pyometra. Endometrial proliferation factors exacerbate uterine innate immune responses. Whereas *Escherichia coli* is the most common organism isolated in up to 90% of cases with a positive culture, genes encoding for adhesins, toxins, and other factors increase virulence of certain strains. A presumptive diagnosis of pyometra is based on clinical signs and laboratory tests, with a confirmatory diagnosis based on B-mode ultrasonography, with or without Doppler ultrasonography, detecting an enlarged, fluid-filled uterus with increased blood flow. Broad-spectrum antibiotics with minimal nephrotoxicity are needed to prevent septicemia, but antibiotics alone will not resolve pyometra. Safest and most effective treatment is surgical removal of infected uterus and ovaries. Medical management of pyometra may be indicated to improve general status of the bitch prior to surgery or treatment in valuable breeding bitches. Protocols using prostaglandin $F_{2\alpha}$ or aglepristone are discussed in detail.

Keywords: Aglepristone, cystic endometrial hyperplasia, *Escherichia coli*, prostaglandin $F_{2\alpha}$, virulence factors

Introduction

Pyometra is a life-threatening bacterial infection of the uterus that affects 20 - 25% of reproductively intact bitches.[1-5] However, incidence of pyometra may be > 50% in certain high-risk breeds, indicating that there may be genetic factors predisposing to development of pyometra.[2] Mean age for pyometra diagnosis in bitches is 7 years (range: 4 months to 18 years).[2]

Etiopathogenesis

Canine pyometra is characterized by an accumulation of leukocytes and exudate within uterine lumen. Although exact temporal and progressive mechanisms underlying pathogenesis are not fully understood, a combination of endocrine, structural, inflammatory and bacterial factors are likely involved in most cases.

Endocrine factors

Pyometra develops during luteal phase; progesterone has a key role for establishment of infection with opportunistic bacteria. During diestrus, progesterone induces endometrial proliferation, endometrial gland secretion, myometrial quiescence and cervical closure.[6-7] Progesterone not only inhibits endometrial bactericidal activity, it also specifically increase the affinity of endometrium to *Escherichia coli (E. coli)*.[8] In addition, repeated exposure to endogenous and/or exogenous progestogens has an important role in pathogenesis of cystic endometrial hyperplasia, which also has a key role in development of pyometra.[9-12]

Structural factors

Cystic endometrial hyperplasia (CEH) results from proliferation of endometrial glands and subsequent endometrial cyst formation. CEH is accompanied by severe changes in endometrial cell proliferation and apoptosis, coinciding with dysregulation in expression of apoptosis regulatory proteins (e.g. Bcl2, Bax).[13] These alterations cause excessive proliferation, combined with deregulation of

apoptosis, which then causes insufficient endometrial regeneration.[13] Role of progestogens in pathogenesis of CEH is exacerbated by estradiol 17_{β}.[6-7] CEH is the most common uterine disease in dogs.[14] Incidence of CEH increases with age from < 4% in bitches under 3 years of age to > 50% in bitches by 7 years.[15] It is important to note that not all cases of pyometra involve CEH.

Normal uterine defence mechanisms against pyometra include: (1) a potent local immune response with leukocytes and antibodies, (2) well-regulated endometrial secretions and (3) myometrial contractions.[7] However, functional patency of the cervix also has an important structural role. With a functionally patent cervix (open pyometra), mucopurulent to hemorrhagic exudate can exit the uterus. When cervix is not patent (closed pyometra), bacterial laden exudate accumulates within uterine lumen, resulting in endometrial atrophy.[16] Closed pyometras are associated with more severe illness compared to open pyometras, but not poorer outcomes as measured by postoperative hospitalization.[3] With respect to incidence, open pyometra occurs more commonly (65 - 75%) than closed pyometra (25 - 35%).[3,5]

Inflammatory factors

Lipopolysaccharide (LPS) from bacterial cell walls elicits a strong inflammatory response. Uterine inflammation in response to LPS is associated with upregulation of endometrial genes related to the innate immune response. In response to this inflammation, endometrial proliferation factors exacerbate the inflammatory reaction.[17] These factors are: (1) insulin-like growth factor (IGF1), (2) endometrial remodelling (via matrix metalloproteinases) and (3) inflammatory response genes (e.g. chemokine ligand 2 (CXCL2), secretory leukocyte peptidase inhibitor (SPLI)). Based on global endometrial transcriptomic profiling by microarray, many genes are upregulated ~ 10 - 77 times in uteruses of bitches with pyometra.[18]

Pyometra can be histologically classified as either hyperplastic or atrophic.[19] Endometrial thickness and ratio of endometrial to myometrial thickness are increased 3-fold in hyperplastic pyometra compared to a normal uterus.[19] Histologically, a uterus associated with atrophic pyometra has severe thinning of endometrial mucosa (with thin cuboidal epithelial cells) with concurrent hypertrophy of myometrium.[19] These 2 forms have differential regulation of inflammatory cytokines and enzymes in the prostaglandin synthetic pathway in the endometrium.[19] For example, a relative fold increase in interleukin 8 (IL8) of 1.82 times occurs in atrophic pyometra compared to hyperplastic pyometra.[19] In addition, expression of cyclooxygenase type-2 (COX2) and prostaglandin F synthase (PGFS) are significantly upregulated (3.75 and 3.15 times in atrophic pyometra compared to hyperplastic pyometra, respectively).[19] This results in greater expression of proliferative Ki-67 marker in endometrial stromal cells in response to the bacterial agent.[13]

During inflammation, COX2 is an important source of prostaglandins and thromboxane A2. COX-2 interacts directly with chemokines that are overexpressed in pyometra (e.g. CXCL8/IL8, CXCL14).[17] Overexpression of CXCL14 contributes to inflammatory cell infiltration into uterine lumen, due to its chemoattractant action in monocytes and natural killer cells.[20] However, CXCL10 has antimicrobial activity against *E. coli*.[21] In stromal cells, CXCL10 may be involved in recruitment and potentiation of T helper 1 response.[22] Because IL8 and CXCL14 are induced by COX2, selective COX2 inhibitors may mitigate inflammatory response during pyometra.[17]

COX2 is also downstream of toll-like receptor (TLR) signalling after activation by endogenous S100 proteins and other stimuli (e.g. LPS). Conserved pathogen-associated molecular patterns synthesized by microorganisms (LPS) are recognized by TLR to initiate an innate immune response. Normal canine endometrial epithelial and stromal cells express TLR4, but both TLR2 and TLR4 are upregulated in pyometra. TLR mediated immune surveillance is an important component in uterine defence mechanisms.[23] Continuous activation of TLR by both endogenous and exogenous stimulus can lead to an exacerbated inflammatory response in pyometra. LPS interacts with lactoferrin (LTF) on the surface of bacteria to activate TLR4 on surfaces of phagocytes and epithelial cells.[17] Both LTF gene and protein are overexpressed in pyometra.[24]

Calcium-binding proteins of the S100 family (e.g. S100A8, S100A9, S100A12) have been identified as endogenous danger-associated molecular patterns (DAMPs).[17] DAMPs are intracellular

molecules released following cell death and are recognized by the innate immune system. S100A8 and S100A9 stimulate production of several pro-inflammatory cytokines (e.g. tumor necrosis factor (TNF), IL6, IL1B, IL8).[17] Another op upregulated gene in pyometra is secretory leukocyte peptidase inhibitor (SLPI) gene.[17] SLPI encodes for an antimicrobial peptide secreted by epithelial tissues. SPLI modulates infection and inflammation by neutralizing LPS and reducing activation of TLRs.[17]

Bacteria

Positive uterine cultures have been obtained during various stages of the reproductive cycle in bitches without reproductive problems.[25] Common bacteria in uteruses of healthy bitches reflect bacterial flora of vagina and cervix.[25] Presence of mixed bacteria in uterus during proestrus and estrus is not uncommon in dogs, presumably due to cervical dilation.[25] Presence of intrauterine bacteria cultured from uterine lumen following elective ovariohysterectomies during diestrus is counterintuitive. In 1 study, > 10% of "normal" diestrous uteruses yielded positive bacterial cultures.[25] Organisms isolated included *Enterococcus* sp., *Bacillus* sp., and undefined gram positive species.[25] Furthermore, ~ 16% of bitches believed to have pyometra have negative bacteriologic findings following ovariohysterectomy.[25] However, there were no attempts to detect anaerobic organisms or isolate other bacteria such as *Mycoplasma* sp., *Leptospira* sp., or *Chlamydia* sp.[25]

With respect to pyometra, *E. coli* is the most common organism isolated in 90% of cases with positive culture.[26-27] However, other bacteria are also cultured (Table 1), with *Enterococcus avium* isolated from the uterus of a dog with an emphysematous pyometra. In > 90% of cases of canine pyometra, only a single bacterial species was cultured.[25] It is important to mention that when > 1 bacterial species is isolated from canine pyometra, *E. coli* is always isolated.[25]

Escherichia coli

Strains of these that are isolated from pyometra are those that normally inhabit canine intestine and those present in urinary tract infections.[28] *Escherichia coli* isolated from canine pyometra is mainly assigned to phylogenetic group B2 and characterized by a high number of

Table 1. Bacteria other than *Escherichia coli* cultured from cases of canine pyometra.[25,72]

Bacteria	Frequency (%)
B-hemolytic *Streptococcus*	15
Citrobacter spp.	10
Unidentified *Enterobacteriaceae*	5.9 - 33.3
Streptococcus spp.	5.1
Klebsiella spp.	4.2
Enterococcus spp.	3.4
Actinomycetaceae spp.	2.5
Staphylococcus spp.	2.5 - 16.7
Pseudomonas spp.	1.7 - 2.0
Undefined Gram negatives	0.8
Undefined Gram positives	0.8
Proteus	0.8 - 16.7

uropathogenic *E. coli* (UPEC) virulence factor (VF) genes and pathogenicity-associated island markers.[28] *Escherichia coli* have VF genes encoding for adhesins (including *fimH*, *papC* and *papGIII*), toxins (including *hlyA/F*, *usp* and *astA*), and other factors (e.g. those involved in escape from host defences and iron uptake).[28] Frequency of VF genes in *E. coli* isolates from canine pyometra are summarized in Table 2. *Escherichia coli* isolates with > 2 adhesin genes imply that a combination of genes confers an

advantage.[28] Strongly virulent strains of *E. coli* (e.g. *nau-b*) contain 7 virulence genes. When strongly virulent strains of *E. coli* are inoculated into female dogs, they produce symptoms of pyometra with an earlier onset and greater severity than weakly virulent strains of *E. coli* (e.g. *nau-i*) with fewer virulence genes.[28] Alpha-hemolysin (*hlyA*) VF gene was detected in 35 - 52% of *E. coli* pyometra cases.[27,29-30] *HlyA* is an RTX pore-forming exotoxin that contributes to virulence of *E. coli* by inducing tissue damage and a compromised early uterine immune response.[27]

Table 2. Virulence genes frequency in *Escherichia coli* isolates from canine pyometra.[28,73]

Gene	Encodes for	Frequency expressed (%)
fimH	adhesin	91.3
irp-2	yersiniabactin	91.3
fim	Type 1 fimbriae	90.9
fyuA	yersiniabactin	82.6
*hly*A	hemolysin	30.4 - 63.6
sfa	S fimbriae	63.6
pap	P fimbriae	63.6
papC	P fimbriae	30.4 - 63.6
papGIII	adhesin	26.1 - 63.6
iroN	salmochelin	56.5
usp	Uropathgenic-specific protein	39.1 - 54.5
traT	Escape from host defenses	47.8
sfaD/E	adhesin	34.8
tsh	adhesin	34.8
cnf-1	toxin	26.1
papE/F	adhesin	21.7
iss	Escape from host defenses	21.7
iutA	aerobactin system	17.4
ompT	Escape from host defenses	17.4
cvaC	Escape from host defense	17.4
hlyF	toxin	17.4
iucD	aerobactin system	17.4
iucC	aerobactin system	13.0
astA	toxin	13.0
papGII	adhesin	4.3

At high concentrations, *HlyA* is able to lyse erythrocytes and nucleated host cells. At low (sublytic) concentrations, *HlyA* can disrupt the immune signalling and cytoskeletal components.[31] Endometrial epithelial cells had high sensitivity to cytotoxic effect of HlyA, suggesting that β-hemolytic *E. coli* induces earlier damage to the epithelial glandular cells than nonhemolytic *E. coli* strains.[32]

Diagnosis

A presumptive diagnosis of pyometra is based on clinical signs and laboratory test results, with a confirmatory diagnosis made with B-mode ultrasonography, with or without Doppler ultrasonography, detecting an enlarged, fluid-filled uterus with increased blood flow.

Clinical signs and laboratory test results

Clinical signs common in pyometra include depression, anorexia, polydipsia/polyuria, vomiting and vaginal discharge.[5,33-34] Anorexia, polydipsia/polyuria, vomiting, and moderate to severe dehydration did not differ significantly between cases of open and closed pyometra.[3] Leukocytosis, with neutrophilia and left shift, lymphopenia and monocytosis are characteristic findings in pyometra, accompanied by normocytic, normochromic regenerative anemia.[5] If present, leukopenia was the most important predictive factor for prolonged postoperative hospitalization, as it was associated with an 18-fold increased risk of peritonitis.[5]

Concomitant cystitis, proteinuria, and hypoalbuminemia usually resolve after treatment of the pyometra, but severe proteinuria that remains may predispose to renal failure.[35] Renal dysfunction is common. Contributing factors to renal dysfunction are: endotoxemia, glomerular dysfunction, renal tubular damage and decreased response to antidiuretic hormone.[35-36] Blood urea nitrogen > 30 mg/dl and creatinine concentrations > 1.5 mg/dl have been associated with death.[33] Hypercholesterolemia, increased serum alkaline phosphatase (ALP) and coagulation impairment have also been reported.[36-38]

Systemic inflammatory response syndrome

In ~ 50% of cases, pyometra can progress into systemic inflammatory response syndrome (SIRS), a form of sepsis and endotoxemia that causes multi-organ dysfunctions.[4,39-41] SIRS is more common in dogs with closed pyometra than an open pyometra.[3] Clinical diagnostic criteria for SIRS are nonspecific but consist of tachycardia and tachypnea, with a similar hemogram as previously described for pyometra. In pyometra cases diagnosed with SIRS, there are increases in blood concentrations of: C-reactive protein (CRP), prostaglandin 15-ketodihydro-$PGF_{2\alpha}$ metabolite, serum amyloid A and haptoglobin. In addition, there were high serum IL8 concentrations in dogs SIRS, suggesting IL8 may contribute to development of systemic disease in dogs with pyometra. Despite being potentially life-threatening, mortality due to pyometra is relatively low (3 - 10%).[5,42]

Ultrasonography

In cases of hyperplastic pyometra, proliferative response can be detected with B-mode ultrasonography as a thickened uterine wall.[15] In addition, increased expression of VEGF-A and its receptors during pyometra cause increased blood flow (both peak systolic flow and end diastolic flow) from reduced vascular resistance.[15] Females diagnosed with CEH and mucometra should be monitored by Doppler ultrasound during diestrus for early pyometra identification, to prevent development of systemic disease.[15]

Treatment

Pyometra is an emergency condition that requires rapid medical and/or surgical intervention to minimize long-term health complications and prevent death. Early intervention increases chances of survival.[43]

Surgical management

Safest and most effective treatment is surgical removal of infected uterus and ovaries, (ovariohysterectomy). Anesthetic risks associated with this surgery are heightened due to a compromised status. Uterus may be large, friable, and prone to rupture, so it is important to handle it carefully. Abdominal cavity should be protected from accidental leakage of purulent material from uterine laceration or uterine tubes/ovarian bursa opening. This is accomplished by packing off uterus with moistened laparotomy pads.[43] Prior to surgery, patient is stabilized with adequate intravenous fluid therapy to correct hypotension (60 ml/kg + % dehydration x body weight/100), hypoperfusion, shock, dehydration, acid-base balance and electrolyte abnormalities, coagulation disturbances, and organ dysfunctions.[44] Intensive postoperative monitoring is essential and in uncomplicated cases, 1 to 2 days of postoperative hospitalization is usually adequate.[43]

Broad-spectrum antibiotics with minimal nephrotoxicity are needed to prevent septicemia (e.g. amoxicillin-clavulanic acid 12.5 mg/kg twice a day). Antibiotics alone for treatment of pyometra will improve general status and may prevent progression but will not resolve the condition. Antibiotics should be continued for $\geq$ 2 weeks.[45] Antibiotic drug selection and route of administration should be based on bacterial culture, sensitivity tests and pharmacokinetics for achieving optimal effect.[43] The initial choice of antibiotics should be effective against the most likely pathogen (*E. coli*) and adjusted after culture and sensitivity results to a narrow-spectrum alternative.[43,46] In 1 study, 90% of E. coli pyometra isolates were sensitive to ampicillin.[47] Intravenous administration may be superior to prevent systemic effects of sepsis.[46]

Medical management

Medical management of pyometra may be indicated to improve the general status of the bitch prior to surgery or in the bitch that cannot undergo general anesthesia for other reasons. In addition, medical management may be indicated for treatment of pyometra in valuable breeding bitches. However, the efficacy of medical management depends on clinical presentation (e.g. stability of patient, patency of cervix) and presence of underlying problems (e.g. cystic endometrial hyperplasia, ovarian cyst or tumor).[48] Goal of medical management is to evacuate uterine contents by dilating cervix and stimulating myometrial contractility.

Protocols using prostaglandin $F_{2\alpha}$ ($PGF2_{\alpha}$) and progesterone antagonists (aglepristone) are described in Table 3. Aglepristone can be used to successfully treat either open or closed pyometras, with cervical opening occurring within 25.8 ± 12.3 hours after initial treatment.[16,48-50] However, $PGF_{2\alpha}$ treatment is contraindicated in closed pyometra due to its potential for forcing purulent material retrograding to uterine tubes through ovarian bursae and into peritoneal cavity or through rupture of uterine wall.[51] Administration of prostaglandin E_2 (either intravaginally or orally) may result in cervical relaxation sufficient to allow $PGF_{2\alpha}$ but this remains to be studied.[45,52] Intrauterine drainage and lavage via transcervical catheterization followed by instillation of intrauterine antibiotics may facilitate recovery in refractory cases.[53-56]

With respect to complications of medical management, ~ 20% of pyometra patients experienced complications, most commonly peritonitis (10 - 12% of cases) followed by chronic pyelonephritis, urinary tract infection, myocarditis, and disseminated intravascular coagulation.[3,5,40,57-59] Persistent proteinuria and urinary protein-creatinine indicate renal disease that requires special attention.[35] Other reported complications include uveitis, intracranial thromboemboli, bacterial osteomyelitis, pericarditis, septic arthritis, incisional swelling, dehiscence, urethral trauma, recurrent estrus, uterine stump pyometra, fistulous tracts, urinary incontinence, septic shock and death.[35,58-59] Severe complications associated with pyometra include sepsis, septic shock, peritonitis, disseminated bacterial infection organ dysfunctions and death.[58,60-61] Proportion of dogs with complications did not differ significantly between open and closed pyometras.[3] Despite this, it is prudent to obtain signed owner consent prior to treatment, regarding potential risks necessary to obtain prior to extra label drug usage.[3] In addition, aglepristone is not currently approved for use in North America.

Prognosis

Following medical management of pyometra with $PGF_{2\alpha}$ or aglepristone, prognosis for survival and fertility is considered guarded to good. Average fertility rate following medical management is 70% (range, 14 - 100%).[43,51,62-64] Fertility rates are even higher in younger (< 5 years) bitches and those that have no other uterine or ovarian pathology.[50] Average reported long-term success (resolution of clinical illness) following medical management of canine pyometra with $PGF_{2\alpha}$ is 86% (range, 46 - 100%).[48-49,54,62-70] Combining $PGF_{2\alpha}$ with cabergoline (a dopamine agonist) results in resolution rates from 83 - 90.5%.[66-67] Resolution rate with aglepristone alone is lower (60%) than with a combination of aglepristone and cloprostenol (84%).[16]

Breeding on subsequent estrus is frequently recommended after medical management of pyometra in attempt to avoid recurrence.[43] Mean recurrence rate following medical management with either treatment is ~ 20% (range, 0 - 85%).[43,49-50,63,71] It is important to mention that medical management of bitches with recurrence has been successful. At least in some breeds, pregnancy is considered to slightly reduce the risk for pyometra recurrence, which points to genetic differences in protective factors.[10,39] However, pregnancy is not completely protective, and developing pyometra during pregnancy has been reported in several cases.[15]

Table 3. Medical management of canine pyometra using prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or aglepristone.

	Mechanism of action	Protocol	Efficacy	Adverse effects
$PGF_{2\alpha}$	$PGF_{2\alpha}$ is both luteolytic and uterotonic. Efficacy of treatment is correlated to repeated administration rather than overall total dose administered. Comparison of uterotonic effect in diestrus bitches of $PGF_{2\alpha}$ at a dosage of 250 µg/kg compared to 50 µg/kg resulted in uterine contractility for 32 and 23 minutes, respectively.[51,75]	$PGF_{2\alpha}$ is most effective when administered in repeated low doses for 8 - 10 days. Administer natural $PGF_{2\alpha}$ at a dosage of 10 - 50 µg/kg SC every 4 - 6 hours.[7,45,68-70] Administer cloprostenol at a dosage of 1 - 3 µg/kg SC every 12 - 24 hours.[62,74]	Following first treatment, copious discharge will be observed coming from vulva for first 48 hours and then will start to decrease. Hemorrhagic discharge may be observed 4 - 5 days into treatment. Evacuation of uterine contents greatly improves bitch's physical condition. Treatment efficacy is confirmed by ultrasonographic clearance of uterine contents 10 - 14 days after first treatment.	Adverse effects are dose-dependent and develop within minutes and last for ~ 1 hour.[62] Adverse effects include hypothermia and shivering, increased frequency of defecation (± diarrhea), hypersalivation and vomiting. Administer atropine (25 µg/kg SC) 15 minutes before $PGF_{2\alpha}$ and walk bitch for 15 - 20 minutes after administration to lessen symptoms. Brachycephalic breeds may also be predisposed to bronchospasm.[45,52] Dosage calculations must be done correctly to prevent serious adverse effects, including hypovolemic shock, ventricular tachycardia and death.[43]
Aglepristone	Aglepristone is a progesterone receptor antagonist that competitively binds progesterone receptors and decreases intrauterine progesterone concentration. Aglepristone does not have direct uterotonic activity.	Administer aglepristone at a dosage of 10 mg/kg SC on days 1, 3, 6 and 9 of treatment.[63] If uterine contents are still visible on day 15, administer another injection on day 15 and on day 30. Results can be improved by giving cloprostenol (1 µg/kg SC) from days 3 - 7.[16]	When a combination of aglepristone and cloprostenol is administered, there was no significant difference in success rates between bitches with open versus closed pyometra.[16]	Adverse effects have not been reported when using aglepristone alone. Vomiting has been reported when used in combination with cloprostenol.[16]

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