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# Selected aspects of endometritis – pyometra complex in dogs – current troubles and treatment perspectives

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## Abstract
Pyometra is the most common gynecological disease in female dogs. It usually occurs in middle age female dogs, usually about two months after the completion of heat. This disease is the accumulation of purulent fluid inside the uterus. Etiology of pyometra is not fully understood. It is assumed, that pyometra is a result of hormonal disorders in the endometrium combined with bacterial superinfection. The diagnosis is based on the interview, clinical examination, additional laboratory tests and ultrasound or x-ray of the abdomen. There are two treatments: ovariohysterectomy and conservative treatment with pharmacological agents for example prostaglandin, aglepriston, antibiotics with a broad spectrum of action. Currently conducted molecular studies have a large influence on the development of the present knowledge on the pathogenesis and course of pyometra, whose conclusions may be used to change the current therapeutic protocols.

**Running title:** *Endometritis – pyometra complex* in dogs

**Keywords:** dog, pyometra, risk factors

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## Introduction

Three forms of *endometritis - pyometra complex* (EPC) are usually characterised: *endometritis chronica, hyperplasia glandularis cystica*, and *pyometra* [1,2]. *Pyometra* is a disease that normally affects non-sterilised bitches of middle and old age but has also been observed in young specimen (even few months old, after first menstruation). It is one of the most commonly diagnosed gynaecological malignancies in dogs. Rottweilers, Border Collie, Golden Retrievers, Labradors, German Shepherds and Bernese Mountain dogs are among the most predisposed breeds [3,4]. Numerous observations have proven that bitches that have never given birth are especially prone to be affected [5,6,7]. The disease causes accumulation of pus like liquid inside the uterus. It occurs in the dioestrus phase of the menstrual cycle, most commonly around two months after menstruation. It can be accompanied by open cervix (with pus outflow), or closed cervix (without visible outflow). Rarely, *pyometra* of the stump of the uterus is also observed [7]. Dow characterises 4 stages of *pyometra* in bitches. Stage I: cystic overgrowth of endometrium; Stage II: invasion of inflamed cells; Stage III: acute endometrial inflammation; Stage IV: chronic endometrial inflammation [8].

## Historical Outline

One of the first mentions of *pyometra* was the report by Aranez et al., which used exploratory laparotomy or amniocentesis (in order to rule out ascites) for diagnostics [9]. Treatment included ovariohysterectomy and parenteral antibiotic administration. Presence of *pyometra* was then often confirmed through dissection. Sandholm et al. noticed that some of the bitches showed the presence of the same strain of *E. coli* in uterus and bladder [10]. They assumed that the specimen must have been affected by subclinical cystitis, causing infection of uterus during early dioestrus phase. However, it is not known if the cystitis was primary or recurring. Borresen figured that *pyometra* is caused by a mixture of hormonal and microbiological factors [11]. Additionally, it can also be caused in an iatrogenic manner, after parenteral progesterone administration. Chafaux et al., in their study, observed the influence of progesterone of *pyometra* progression [12]. They presumed that high levels of this hormone, or its prolonged expression, can have significant effect on the occurrence of the disease. Douglas described marsupialisation as an effective method of *pyometra* treatment [13]. It was performed through making a small incision in the abdomen and sewing the edges of the incised uterine horn to the wound. This action aimed to evacuate the pus that filled the uterus, as well as stabilisation of the patient's state. After 48 hours, routine ovariohysterectomy was performed.

## Physiology

Dogs belong to the group of animals that are seasonally monoestrus. This occurrence is individual for every bitch. The reproductive cycle is constructed out of 4 phases: *proestrus, oestrus, dioestrus and anoestrus* [14,15]. *Proestrus* lasts from 5 to 20 days. It is characterised by changes in the external sexual organs, above all blood or blood-mucus outflow from the reproductive tract [14]. In this phase, under the influence of FSG, the follicles start to grow and mature, appearing small (1-3mm) and hypoechoic in ultrasound [16]. In the same time, the level of oestradiol produced by granulosa cells gradually increases (initially 5-15 pg/ml, peak 40-120 pg/ml), to decrease in the end of this phase. Progesterone levels rise in the final phase (1-3ng/ml) [14,17]. This occurrence is described as preovulatory follicle luteinisation. During pap test, may intermediate cell can be observed, as well as increasing amount of surface and nucleus free cells [15]. In this phase the bitch attracts male attention but is not ready for copulation. *Oestrus* lasts around 5-15 days [14]. It starts with LH surge. Progesterone levels increases intensely (10-25 ng/ml). After 24-72 hours after LH surge, ovulation occurs. Keratinized cells dominate the pap tests (above 80%). In this period the bitch is ready for copulation [15]. Ultrasound shows hypoechoic follicles (>4mm) [16]. *Dioestrus* lasts about 50-80 days [14]. It is the luteal phase, in which progesterone is the main ovarian hormone (in physiological state). *Corpus luteum* can be observed in the ovary. Intermediate cells dominate in pap test, with some neutrophils present in the first days of that phase. Progesterone levels do not significantly vary between pregnant and non-pregnant bitches [14,15]. *Anoestrus* lasts between 80-240 days [14]. It is the phase of hormonal silencing. The levels of oestradiol and progesterone are barely detectable. The ovary lacks functional structures. Singular intermediate cells can be observed in the pap test [15].

## Pathogenesis

Aetiopathogenesis of *pyometra* is not fully discovered. It is assumed that it is caused by hormonal disturbances in the endometrium, combined with bacterial infection. Progesterone, which stimulates the secretion in uterine glands, is the dominating hormone in *dioestrus.* Additionally, elongated menstruation or ovarian cysts that produce oestrogen stimulate endometrial proliferation, which results in delayed evacuation of these glands [18,19]. Progesterone also causes the decrease in local immune response, which increases the chance of bacterial infection. E. coli are the most commonly isolated bacterial types [20–24]. Bigliardi et al. isolated 13 different serotypes of these bacteria [20]. Infections with *Streptococcus canis, Enterobacter cloacae, Proteus sp., Klebsiella sp., Pseudomonas sp.* also occur. In some cases, no bacterial infections are found.

## Diagnostics

Clinical symptoms of *pyometra* are non-specific. They include: apathy, lowered appetite, with sporadic vomiting, diarrhoea and increase in abdominal volume. In the case of *pyometra* accompanied with vaginal pus outflow, polyuria, polydipsia, weakness of hindlegs and dehydration is also observed. Body temperature increases. Morbidity is around 4%, according to Hagman et al. [4], and around 3-10% according to Jitpean et al. [25]. Diagnosis is given based on: interview, clinical exam, laboratory tests, cervical smear and image diagnostics. In the interview, specific questions such as: is the dog sterilised, when was the last menstruation, has the bitch ever given birth and if the owners noticed vaginal pus outflow; should be included. In clinical exam, increased body temperature, vaginal pus outflow, abdominal pain and, in advanced cases, paleness of mucus membranes and petechia are symptomatic to the disease. Fidler et al. observed that *pyometra* occurred more commonly in bitches that exhibited irregular menstruation, with less prevalence in specimen that went through false pregnancies [26]. In the case of open cervix *pyometra,* pap smear is an easily performable and interpretable test for identification of the disease. It is worth noting that in closed cervix *pyometra* the pap smear results will not be abnormal. Laboratory results should indicate leucocytosis and neutrophilia. In severe cases (with advanced septicaemia), leukopenia is noted, which is synonymous to bad prognoses. In some cases, most commonly in open cervix *pyometra,* leukocyte counts might be normal. Anaemia (normocytic, normochromic) and thrombocytopenia, which might be the result of toxic bone marrow damage, are also observed. Hyperproteinaemia (caused by gamma globulin increase) with hypoalbuminemia (linked to loss of albumins in the kidneys) also commonly occurs. Disturbances in liver and kidney functions are often present, indicated by loss of ALT and AST activity, as well as fall in creatinine and urea concentrations. Performing UPC might also be helpful, as a marker of kidney damage [5,7,25,27,28]but its prevalence and clinical relevance are not well characterized. OBJECTIVES To define which subset of dogs with pyometra has clinically relevant kidney injury by quantification of proteinuria; light, immunofluorescence, and electron microscopic examination of kidney biopsy specimens; and measurement of urinary biomarkers. ANIMALS Forty-seven dogs with pyometra. Ten clinically healthy intact bitches of comparable age. METHODS Prospective study. Routine clinicopathological variables including urinary protein to creatinine ratio (UPC. Dąbrowski et al. found that, during *pyometra,* serum levels of acute-phase protein, which play a major role in immunological response, rise significantly [29]. They have also noted major increase in serum KYNA in bitches with diagnosed *pyometra*, suggesting its potential role as a marker of that disease [30]. They have also analysed serum IGF-1 concentrations, as potential *pyometra* marker [31]. However, these studies did not yield meaningful results. Ultrasounds and x-rays are most commonly used in image diagnostics. Abdominal x-ray shows enlarged uterus. Tissue saturation corresponds to that of fluid/soft tissues. It is important to note that these results need to be differed from pregnancy (foetal skeleton saturation is only visible around day 45) and postpartum period [28]. Abdominal ultrasound presents clearer results. It allows for estimation of the amount of fluid in the uterine lumen. Additionally, it permits the evaluation of the accompanying peritoneal inflammation [20]. In young bitches in good clinical state, with non-advanced *pyometra*, that are set to undergo conservative treatment, it is recommended to rule out the presence of ovarian cysts. In rare cases, usually accidental, *pyometra* can be recognized through CAT or MRI scan. In the future, molecular tests, such as estimation of specific gene expression with the use of microarray, could become useful for monitoring the disease and determining the prognosis for individual patients [32,33].

## Clinical Approaches

Untreated *pyometra* leads to death of the animal. The most common treatment is ovariohysterectomy [25]. However, in some examples (young bitches used for breeding or animals in bad clinical state) conservative treatment can be applied. There are different approaches to this kind of treatment. Nelson et al. used natural F2α prostaglandin, administered parenterally in 0,1-0,5 mg/kg of body mass, once a day till the vaginal outflow stops (max 5 days) [34]. They observed side effects, such as: excessive salivation, vomiting and diarrhoea. To prevent these, they have recommended walking the dog 30 minutes after medicine administration. It is worth noting that this drug is not registered for treatment of dogs and should only be used in extraordinary situations, after obtaining written consent of the owner. Gabor et al. state that intravaginal administration of natural F2α prostaglandin (150mg/kg of body mass, or 0,3ml/10kg of body mass) twice a day might be an effective *pyometra* treatment [35]. In the same time, general antibiotic treatment with the use of amoxicillin (15mg/kg of body mass, every 48h) and/or gentamycin (4mg/kg of body mass, every 24g) should be applied. In most of the cases, the animals came back to health after 3-12 days. Side effects of prostaglandin administration were not observed. Trasch et al. achieved 92,3% treatment rate with the use of aglepristone [36]. Relapse can be limited by elimination of bitches that exhibit ovarian cysts and uterine changes, from conservative treatment. However, Gürbulak et al. think that effective treatment involves aglepristone administration in day 1 (day of diagnosis), as

well as days 2, 7 and 14, in a dost of 10mg/kg, combined with intra-uterine administration of antibiotics, based on antibiogram results [37]. Hormonal activity was exhibited by 58,3% bitches that have undergone treatment, 3 out of 14 became pregnant. Fieni used another schematic of the therapy [38]. Aglepristone was administered in a single dose of 10mg/kg of body mass, at day 1, 2 and 8 (from the diagnosis). Between 3rd and 7th day of treatment, cloprostenol was additionally administered. Bitches with high body temperature and dehydration were subjected to additional treatment: liquid therapy with the use of Ringer fluid with lactates, as well as antibiotic therapy with the use of amoxicillin and clavulonic acid for 1-5 days. In some bitches, aglepristone was also administered on days 14 and 28 of therapy. Cervix opened in 48 hours after aglepristone administration. 84% of treated dogs returned to health (evaluated 90 days after diagnosis). Author recommends that treatment in bitches that did not exhibit symptoms of kidney or liver disfunction. Ros et al. proposes aglepristone therapy, using doses of 10mg/kg of body mass in the day of diagnosis, day 2, 7/8, 14/15, and every 7-8 days until the end of treatment [39]. Additionally, he has applied antibiotic therapy with the use of: enrofloxacin, amoxicillin, amoxicillin with clavulanic acid, marbofloxacin, potentiated sulphonamides, or metronidazole. The efficacy of that therapy was around 75%. Contri et al. found that aglepristone administration in a dose of 10mg/kg of body mass in day 1, 3, 6 and 9 of treatment is even more effective (100% of dogs treated) than in days 1, 2 and 7 (88,5% treatment rate) [40]. In both cases antibiotic therapy was also used (amoxicillin with clavulanic acid, 20mg/kg of body mass/day), together with liquid therapy with the use of Ringer fluid with lactates. Prostaglandin F2-alpha (250μg/kg) can also be used for the therapy, with subdermal administration every 12 hours, until the return of uterus to the right size [7,41]. The treatment usually lasts for 3-5 days. Studies on the efficiency 3rd generation gonadotropin use in bitches (330 μg/kg), at the day of diagnosis, combined with orally administered antibiotics (amoxicillin with clavulanic acid, 12mg/kg every 12 hours). After 3 days, improvement in health was noted. No side effects were observed. However, the research has been conducted on a small group of 4 dogs and requires further validation [42]. The most common complications of *pyometra* are: sepsis and septic shock, peritoneum inflammation and bleeding [25]. The risk of heart ischaemia during surgical treatments, which may cause an increase in mortality after the procedure of ovariohysterectomy, also needs to be noted [43]. Checking blood urea levels after the surgery might be useful for accurate prognosis. If the levels increase after the procedure, the prognosis is poor, as kidney function has been impaired [28]. To estimate the stage of wound healing and recovery after OVH procedure, serum IL-6 and IL-10 levels can be used. According to Dąbrowski et al., they were much higher in disease affected bitches and have started to normalize after around 3 days after the OVH procedure [44]. The serum levels of endotoxins coming from gram-negative bacteria can also be used for accurate recovery prognosis. It was noted that the severity of the disease was proportional to their blood levels, with prognoses worsening and the risk of sudden death higher when the levels were high [45]. Hagman et al. investigated the levels of lactates in bitches affected with *pyometra* [46]. The results showed that only 1% of dogs had elevated blood lactate levels, which rules out their use in predicting complications of the disease. Troponin I levels were also examined before and 1 day after the ovariohysterectomy procedure, in bitches affected with *pyometra*. 28% of dogs before surgery and 39% of those that underwent the procedure had elevated levels of that protein. Its levels rose in some bitches after the procedure while falling in others, which makes its diagnostic significance doubtful [46].

## Perspectives

*Pyometra* in bitches is a disease linked to activation and proliferation of specific inflammatory cells, as well as activation of specific biochemical pathways associated with immune response. Through the years, molecular biomarkers were identified in the endometrium, allowing identification of genes upregulated during that disease [5,33]which is accompanied by bacterial contamination of the uterus, is defined as a complex disease associated with the activation of several systems, including the immune system. The objective of the study was to evaluate the gene expression profile in dogs with pyometra compared with those that were clinically normal. The study included uteri from 43 mongrel bitches (23 with pyometra, 20 clinically healthy. In 2012, over 800 upregulated genes were identified. Many of them are linked to inflammatory response, e.g. chemokines, cytokines, metalloproteins, collagenases, TLR2 and TLR4 [5]where approximately 90% of the dog population is intact (not neutered. Bukowska et al., with the use of expression microarrays, analysed 17138 genes, out of which 1360 were upregulated, 1005 were downregulated and 14713 didn't show significant change [33]. 264 genes responsible for inflammatory response were also selected. In 98 of them the expression increased, with the levels decreasing in 10 transcripts. 23 genes with of higher expression were brought to focus, encoding IL-8, IL-1β, IL-18RAP, IL-1α, IL-6, IL-1RN proteins. These interleukins are directly linked to activation of inflammatory response. Increase in transcription levels of Cox-1, Cox-2, PGFS I mPGFS-1 was also described in *pyometra*, in comparison to *dioestrus* and *anoestrus* phases [47]. Karlsson et al.

noted the increase in TNF-α, IL-7, IL-15, IL-18 and IL-8 [48]. Silva et al. concluded that during *pyometra* the expression of TLR2 and TLR4 increases in the endometrium. It is most probably the result of stimulating action of lipopolysaccharides and *E.Coli* [49]the most frequent endometrial disorder in the bitch. Toll-like receptors (TLRs. Additionally, acute-phase proteins might be used to differentiate *pyometra* from other diseases that cause uterine fill up, identify sepsis affected patients, as well as to predict complication and extended hospitalisation [50]. In serum of *pyometra* affected bitches, significant rise in IL-8 levels was observed. These levels were higher in bitches with moderately advanced symptoms [51]. In 2012, expression of TLR genes and proteins in different phases of menstrual cycle in bitches was examined. Significant upregulation of TLR1-7 and 9 was noted in late *dioestrus* and *anoestrus.* Activation of these gens plays and important role in invoking the inflammatory response in the uterus [52]. All that research aimed to identify specific markers for identification and clinical treatment of *pyometra.*

## Conclusions

*Pyometra* is still one of the most commonly recognized gynaecological malignancies in bitches. Nevertheless, it is still full of unknowns. Recognizing of detailed, molecular pathogenesis would most likely ease early identification and prevention of that disease. Application of genetic and molecular studies gives particular perspectives, allowing to determine genes responsible for *pyometra* development. Currently, increasing consciousness of owners, as well as highly developed ultrasound diagnostics, allows for even earlier diagnosis of *pyometra* and monitoring of *dioestrus* phase. More and more methods of conservative treatment also appear, giving hope for treatment of breeding dogs without loss of their fertility.

## Ethical approval

The conducted research is not related to either human or animal use.

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**Conflict of interest statement**

The authors declare they have no conflict of interest.

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