Research Article

Bacterial Agents Determining Canine Pyometra and Virulence Factors Related to Pathogenesis

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Abstract

Pyometra is a pathology that frequently occurs in the reproductive tract of bitches, whose pathogenesis consists of inflammation of the uterus with accumulation of purulent fluid. This condition can affect bitches of different ages, but is more common in the adult phase due to the luteal phase of the estrous cycle and in the diestrus phase due to hypertrophy or hyperplasia of the endometrium, which is stimulated by progesterone. The objective of this study was to identify the main bacterial agents involved in pyometra in bitches, as well as the virulence factors related to its pathogenesis, in order to provide a discussion on the pathogenesis involving hormonal-bacterial interaction and the severity of endotoxemic conditions due to the use of antibiotics. Progesterone promotes the facilitation of bacterial invasion through the virulence factors pap, sfa, aer and cnf; and the increased possibility of recurrences in older female dogs, since the luteal phase repeats itself several times throughout the animal's life. It is important to highlight that different serotypes of Escherichia coli have been associated with pyometra in female dogs and that a heat-labile virulence factor found in all serotypes is responsible for the binding of the bacteria to the brush border of the endometrium during the luteal phase, which facilitates the colonization of the uterus by this bacterial agent. The present study corroborated the importance of *Escherichia coli* as the main etiological agent of canine pyometra, as well as indicating the expression of its virulence factors for its pathogenesis.

Keywords: *Escherichia coli;* Endotoxin; LPS; SIRS; Endometrium

Introduction

Pyometra is a very common pathology of the canine female genital tract, characterized by inflammation of the uterus with the accumulation of exudate, which can occur at different ages but is more predominant in adult bitches, occurring in the luteal phase of the estrous cycle. During diestrus, there is hypertrophy or hyperplasia of the endometrium stimulated by progesterone, which facilitates bacterial invasion [21]. This repetition of the luteal phase that occurs several times throughout the lives of female dogs represents an important factor for the higher frequency of this disease in older animals [1].

Due to the body's responses to estrogen during estrus, followed by periods of progesterone dominance that stimulates the proliferation of the endometrium, there is a decrease in myometrial contractions, an increase in the secretion of uterine glands, and a sterile fluid can accumulate in the uterine lumen, creating an environment conducive to the migration of bacteria from the normal vaginal flora to the uterus, facilitating the occurrence of pyometra [16].

The bacteria most frequently found in cases of pyometra is *Escherichia coli*, which normally is part of the vaginal flora. This bacterium has the ability to adhere to progesterone-stimulated receptors in the myometrium and endometrium through the local specific antigen pathway [6]. Different serotypes of *E. coli* have been associated with pyometra, and it is believed that a thermolabile antigenic factor found in all types is responsible for the bacteria's binding to the brush border of the endometrium during the luteal phase, which would facilitate the colonization of the uterus by these bacteria [16].

Pyometra can be classified as open or closed, with the main clinical signs of the open type potentially including serosanguineous vaginal discharge, fever, apathy, and vomiting, as the estrogenic influence causes the cervix to open, allowing the entry of bacteria from the normal vaginal microbiota into the uterine lumen. In closed pyometra, the main clinical signs are abdominal distension and painful sensitivity, polyuria, lethargy, depression, polydipsia, anorexia, hyporexia, dehydration, and vomiting [12]. The clinical signs are variable and these changes are more severe in closed-cervix pyometra, which can progress to endotoxemic shock and death [14].

According to Feldman and Nelson [6], in cases of open pyometra, a classic sign to be observed is vaginal discharge, which helps establish the diagnosis more quickly and accurately. On the other hand, in the closed version, the female often finds herself with her health severely compromised, leading to a late diagnosis due to the greater complexity in identifying the condition, since bitches with closed pyometra do not present vaginal secretions and rarely show signs of polyarthritis secondary to bacteremia and joint infection, which characterizes closed cervix pyometra as the more severe form, due to the higher risk of developing endotoxemia.

According to Lopez [9], the diagnosis of pyometra consists of a combination of anamnesis, physical, laboratory, and imaging examinations of the uterus and ovaries, with this pathology frequently seen in the clinical routine of small animals, whose aggressiveness is associated with the type of microorganism involved, where the severity and possibility of death are mainly linked to endotoxemia cases caused by pathogenic bacteria.

The objective of the study was to identify the main bacterial agents involved in canine pyometra, as well as the virulence factors related to its pathogenesis, in order to provide a discussion on the pathogenesis involving hormonal-bacterial interaction and the severity of endotoxemic conditions in the face of antibiotic use.

Methodology

The study was conducted based on an exploratory bibliographic research using the scientific databases SciElo, PubMed, CAPES, LILACS, MEDLINE, and Google Scholar. For the search, a publication time frame from 1996 to 2021 was established, using the following keywords: pyometra, pathology, infection, endotoxemia. Articles addressing the topic of pyometra and its pathogens and complications in the medical clinic of bitches, and that established a relationship with risk factors and determinants for the occurrence of these conditions, were included in the study, totaling 23 articles selected for obtaining results and drafting the discussion.

Results and Discussion

The uterus of female dogs is a sterile environment, but conducive to the colonization of microorganisms, which, due to the increase in serum progesterone levels, triggers susceptibility to the development and establishment of bacteria in the uterus, promoting the infectious process of pyometra, directly influenced by hormonal imbalance, associated with the virulence factor of the bacteria involved in this pathology [9,15].

The elevated levels of progesterone that occur during the diestrus period stimulate endometrial proliferation and create an ideal environment for bacterial adhesion and growth, while simultaneously leading to the cessation of leukocyte activity and a decrease in myometrial contractility. Although it is common for Cystic Endometrial Hyperplasia (CEH) to predispose to pyometra, there are also cases of the pathology without a previous occurrence of CEH, which generates discussions regarding the classification of the complex cystic endometrial hyperplasia, that is, pyometra as two different pathologies [3].

There is a consensus among authors that pyometra is the result of the interaction between hormones, with progesterone being the main one, and bacteria. However, even with numerous studies on the subject, pyometra syndrome still remains a challenge for most professionals, and it is not possible to assert that the hormonal-bacterial interaction is the only predisposing factor for this pathology [10,22,23].

In a study conducted by Gorricho and Campos [7], about 900 female dogs of various breeds and mixed breeds affected by pyometra were observed, showing that the hormonal factor influences more than the racial factor in the onset of this disease; however, there are studies that disagree with this theory. In a study conducted in Sweden, Egenvall [4] observed that breeds such as Collie, Rottweiler, Cavalier King Charles Spaniel, Golden Retriever, Bernese Mountain Dog, and English Cocker Spaniel are more predisposed to developing pyometra. Another interesting factor to observe is that nulliparous bitches present higher risks in the development of pyometra, compared to primiparous or multiparous animals, making castration recommended as a preventive measure in cases where the animal will not be used for reproduction [8,20].

According to Vinhas [19], the main etiological agent isolated from the uterine content of pyometra is *Escherichia coli*; however, *Staphylococcus* spp., *Streptococcus* spp., *Bacillus* sp., *Clostridium perfringens, Corynebacterium* sp., *Enterococcus* sp., *Haemophilus* sp., *Klebsiella, Pasteurella*, *Pseudomonas,* Proteus, among others, are also isolated with some frequency.

Despite being the most commonly isolated agent in pyometra content, *E. coli* is not part of the normal bacterial microbiota of the vaginal canal, even though this bacterial type has a great affinity for the endometrium and myometrium, which makes it difficult and even impossible to eliminate by the local defense system. On the other hand, the other bacteria are also easily isolated throughout the genital tract of healthy females, indicating that they are part of the population of bacteria that participate in the infection only as opportunists, with the main ones being *Staphylococcus aureus, Pseudomonas* spp, *Streptococcus* spp, and *Proteus* spp [12].

One of the reasons why the bacterium *E. coli* stands out as one of the most frequent etiological agents of pyometra is due to its virulence factors that facilitate its development in the uterus of bitches. The main virulence factors are the ability to adhere to the epithelium via the P fimbriae, the factors associated with urinary tract infections (pap) and the determination of enteropathogenicity (sfa), afimbrial adhesins (afa), aerobactin (aer), the production of hemolysins (hly), and the cytotoxic necrotizing factor (cnf) [13,17]. In a study conducted by Coggan [2], 23 strains of *E. coli* were evaluated for the presence of the virulence factors pap, sfa, aer, and cnf. Among these 23 strains, 9 (39.13%) were positive for pap, 16

(69.56%) were positive for sfa, 4 (17.4%) were positive for aer, and 2 (8.7%) were positive for cnf, which reinforces that the ability to adhere via fimbriae is an important factor for infections caused by this bacterium.

The bacterium *E. coli* has lipopolysaccharide endotoxins, also called LPS, in its cell membrane, which remain chemically stable and biologically active as long as the pathogen is alive. However, when it dies, the endotoxin is released, leading to cases of clinical endotoxemia. In this case, clinical signs may worsen with the use of antibiotics, as they promote bacterial mortality, which can be lethal for the animal if the LPS dose reaches 0.7 ng/mL [5].

The Systemic Inflammatory Response Syndrome (SIRS) is one of the major concerns related to pyometra, as the inflammatory reaction triggered by the body in response to bacterial infectious assaults is directly associated with the morbidity of this disease, as demonstrated by Trautwein's study [18], in which a large portion of the animals that died (69.3%) presented SIRS, and when associated with a closed cervix status, these values increase by 100%. Therefore, a swift diagnosis is necessary for the precise determination of the disease's progression in order to prevent the animal's death.

Analyzing yet another study conducted by Trautwein [18], 15 bitches with a clinical diagnosis of pyometra underwent surgical treatment of ovariosalpingohysterectomy combined with fluid therapy, antibiotic therapy, and postoperative clinical follow-up. In this study, the material collected from the bitches' uterus was directly inoculated onto two blood agar plates, one incubated in aerobic conditions for 48 hours and the other in anaerobic conditions also for 48 hours. The culture results were similar, with *E. coli* being the most prevalent bacteria, followed by *Streptococcus* spp. The appearance of SIRS was associated with pyometra, where the majority of the animals analyzed presented this condition and two died.

Similarly, in a similar study conducted by Medeiros [11], 35 animals with a confirmed diagnosis of pyometra were observed and underwent ovariohysterectomy. Antibiotics were tested on culture plates containing blood agar in order to evaluate which drug would be more effective in minimizing the effects of a possible endotoxemia caused by bacteria in pyometra. The bacteria most

frequently found in this study was also *E. coli*, and the most effective antibiotics against this pathogen were amoxicillin potentiated by clavulanate and norfloxacin. Another 19 antibiotics were used in the test for gram-negative bacteria and 15 for gram-positive.

Given the above, virulence factors are determinants for the occurrence and severity of canine pyometra, and *E. coli* is potentially pathogenic, having been frequently incriminated in clinical cases. The incidence of pyometra in female dogs is high, with the disease recognized as one of the most common causes of illness and death in this animal species [2].

Conclusion

There is an agreement among authors that *Escherichia coli* is the most recurrent bacterial agent found in bitches with pyometra, followed by *Staphylococcus* spp. This means that the diagnostic and prognostic evaluation of canine pyometra is of great importance to prevent the clinical progression of the disease, which, with the aid of bacteriological and imaging tests, makes it possible to detect potential complications early and even predict the morbidity and mortality of the disease in each case. In cases where the surgical procedure of ovariosalpingohysterectomy is not indicated, performing a culture and antibiogram of the pyometra isolate is essential for choosing the most appropriate medication for each infectious agent, thereby inhibiting the indiscriminate use of antimicrobials, which can lead to resistance of the agents to certain antibiotics.

Bibliography

1. BALTAZAR FN., *et al*. "Perfil de suscetibilidade a antimicrobianos de bactérias isoladas da secreção uterina de cadelas com piometra atendidas em hospital veterinário localizado em São Paulo, SP, Brasil, no período de 2010 a 2015".

2. COGGAN J A., *et al*. "Estudo Microbiológico De Conteúdo Intrauterino De Cadelas Com Piometra E Pesquisa De Fatores De Virulência Em Cepas De Escherichia Coli". *Arquivos do Instituto Biológico* 71 (2004): 1-749.

3. CONRADO. Francisco de Oliveira. Aspectos clínico-patológicos da piometra, Porto Alegre: UFRGS, (2009).

4. EGENVALL., *et al*. "Breed Risk of Pyometra in Insured Dogs in Sweden". *Journal of Veterinary Internal Medicine* 15.6 (2001): 530-538.

5. FELDMAN E C. "O complexo hiperplasia endometrial cística/piometra e infertilidade em cadelas". In: ETTINGER, S. J.; FELDMAN, E. C. Tratado de Medicina Interna Veterinária. 5. ed. Rio de Janeiro: Guanabara Koogan, v. 2, 2004.

6. FELDMAN EC and NELSON RW. "Canine and feline endocrinology and reproduction". 2a Edição. Phyladelphia: W.B Saunders Company, (1996).

7. GORRICHO CM and CAMPOS AGD. "Ocorrência de piometra em cadelas atendidas nas clinicas veterinárias no município de Ituvera/SP no primeiro semestre de 2011". *REVISTA CIENTÍFICA ELETRÔNICA DE MEDICINA VETERINÁRIA* 9.18 (2012) 1-19.

8. LIMA LRS. Piometra em Cadelas, São Paulo, Faculdades Metropolitanas Unidas, (2009).

9. LOPEZ T V., *et al*. "Perfil de sensibilidade antimicrobiana de bactérias isoladas, de piometra em cadelas, frente a gentamicina de infusão uterina (Gentrin®)". *Research, Society and Development* 10.7 (2022).

10. MARTINS Danilo Gama. "Complexo Hiperplasia Endometrial Cística/Piometra Em Cadelas: Fisiopatogenia, Características Clínicas, Laboratoriais E Abordagem Terapêutica". Faculdade de Ciências Agrárias e Veterinárias – Unesp, Campus de Jaboticabal – SP, (2007).

11. MEDEIROS Lylian Karlla Gomes de. "Estudo microbiológico de cadelas submetidas à ovariohisterectomia: analise da antissepsia com ou sem banho prévio e identificação e de bactérias causadoras de pyometra". Universidade Federal de Campina Grande, Centro de Saúde e Tecnologia Rural (2015).

12. OLIVEIRA R G de., *et al*. "Piometra em cadela com complicação renal". *Ciência Animal* 29.1 (2019): 135-145.

13. OLIVEIRA Raquel Peres de. "Virulence factors and genetic similarity of escherichia coli isolated from uterus and urine in female dogs with and without pyometra". 2013. 89 f. Dissertação (Mestrado em Ciências Agrárias) - Universidade Federal de Uberlândia, Uberlândia (2013).

14. SANCHES F C da S., *et al*. "Avaliação bacteriológica uterina de cadelas com Piometra". *Revista Brasileira de Higiene e Sanidade Animal* 9.1 (2015): 111-121.

15. SAPIN., *et al*. "Patologias Do Sistema Genital Feminino De Cães E Gatos". *Science and Animal Health* 5.1 (2017): 35-56.

16. SILVA Efrayn Elizeu Pereira da. Piometra canina. Botucatu, (2009).

17. STENSKE K A., *et al*. "Prevalence of urovirulence genes cnf, hlyD, sfa/foc, and papGIII in fecal Escherichia coli from healthy dogs and their owners". *American Journal of Veterinary Research, Chicago* 70.11 (2009): 1401-1406.

18. TRAUTWEIN L G C., *et al*. "Piometras em cadelas: relação entre o prognóstico clínico e o diagnóstico laboratorial". *Ciência Animal Brasileira Goiânia* 18 (2017): 1-10, e-44302.

19. VINHAS M C C de V., *et al*. "Ocorrência De Acinetobacter Haemolyticus Em Piometra – Relato De Caso". *Revista de Agroecologia no Semiárido (RAS) - (Sousa - PB*) 4.3 (2020): 90-94.

20. VIOLA D D. Piometra em Cadelas, São Paulo, Faculdades Metropolitanas Unidas, (2003).

21. VOLPATO R., *et al*. "Caracterização microbiológica e perfil de resistência das bactérias isoladas do útero de cadelas com piometra aberta e fechada". *Revista portuguesa de Ciências veterinárias*, RCPV, 109 (2014): (591-592) 100-104.

22. VOLPATO R., *et al*. "Imunoistoquímica de útero e cérvice de cadelas com diagnóstico de pyometra". *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* 64.5 (2012).

23. WEISS RR., *et al*. "Avaliação histopatológica, hormonal e bacteriológica da piometra na cadela". *Archives of Veterinary Science* 9 (2004): 81-87.