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Rafael Gariglio Clark Xavier

CLINICAL, EPIDEMIOLOGICAL AND ETIOLOGICAL FEATURES OF CANINE PYOMETRA

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Rafael Gariglio Clark Xavier

CLINICAL, EPIDEMIOLOGICAL AND ETIOLOGICAL FEATURES OF CANINE PYOMETRA

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Dr.(a). Rodrigo Otávio Silveira Silva (Orientador)
Dr.(a). Jordana Almeida Santana
Dr.(a). Leonardo Borges Acurcio
Dr.(a). Fernanda Morcatti Coura
Dr.(a). Luiz Eduardo Duarte de Oliveira

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ABSTRACT

Pyometra is the most common reproductive disease in bitches and it is characterized by a bacterial infection in the uterus with clinical manifestations ranging from purulent vulvar discharges to life-threatening systemic manifestation. *Escherichia coli* stands out as the most frequent pathogen involved, being reported in up to 90% of cases. Despite its great clinical relevance, the pathogenesis of the disease is still poorly understood. Therefore, the aim of this study were: (1) to characterize *E. coli* isolates from uterine contents and feces of bitches affected by pyometra and feces from healthy dogs consuming two different diets; (2) determine the genetic similarity of *E. coli* isolates from the uterine contents and feces of two cohabiting bitches that were diagnosed with pyometra in the same period; (3) assess whether there is an association between bacterial pathogenicity, endometrial histological changes and clinical prognosis in canine pyometra. *E. coli* strains belonging to the B2 phylogroup and positive for virulence factor genes associated mostly with adhesion predominated in the uterine content and rectal swabs of dogs with *E. coli* pyometra. Interestingly, a lower growth rate of *E. coli* from the B2 phylogroup was observed in dogs fed a raw-meat based diet compared to those fed commercial dry feed. These results suggests most cases of *E. coli* pyometra are caused by strains from phylogroup B2, and also indicates that diet can influence intestinal colonization by such strains. The simultaneous occurrence of pyometra in two cohabitant bitches underwent a depth investigation due to the hypothesis of transmission between these animals. Both whole-genome-multilocus sequence typing and single-nucleotide polymorphism analysis supported the hypothesis that the isolates from the uterine content of both animals and from the rectum of one were clonal. This finding confirmed, for the first time, the transmission of *E. coli* associated with pyometra between two animals. Clinical data, histopathological alterations and microbiological findings of dogs with pyometra (n=39) were analyzed in order to assess possible associations. There was an association between the detection of *papC* in *E. coli* isolates and higher necrosis scores. Additionally, the score of necrosis was positively associated with the length of hospitalization, with each point increase in the necrosis score leading to two more days of hospitalization. These results suggest that *papC*-positive *E. coli* plays an important role in the severity of pyometra in dogs.

Keywords: *Escherichia coli*, ExPEC, transmission of pyometra, bacterial pathogenicity.

RESUMO

A piometra é a doença reprodutiva mais comum em cadelas e caracteriza-se por uma infecção bacteriana no útero com manifestações clínicas que variam desde descargas vulvares purulentas até manifestações sistêmicas com risco de vida. *Escherichia coli* destaca-se como o patógeno mais frequentemente envolvido, sendo relatado em até 90% dos casos. Apesar de sua grande relevância clínica, a patogênese da doença ainda é pouco compreendida. Portanto, os objetivos deste estudo foram: (1) caracterizar isolados de *E. coli* de conteúdo uterino e fezes de cadelas afetadas por piometra e fezes de cadelas saudáveis com duas diferentes dietas; (2) determinar a similaridade genética de isolados de *E. coli* do conteúdo uterino e fezes de duas cadelas coabitantes que foram diagnosticadas com piometra no mesmo período; (3) avaliar se existe associação entre patogenicidade bacteriana, alterações histológicas endometriais e prognóstico clínico na piometra canina. Estirpes de *E. coli* pertencentes ao filogrupo B2 e positivas para genes de fator de virulência associados principalmente à adesão predominaram no conteúdo uterino e suabes retais de cadelas com *E. coli* piometra. Curiosamente, uma menor taxa de crescimento de *E. coli* do filogrupo B2 foi observada em cães alimentados com uma dieta à base de carne crua em comparação com aqueles alimentados com ração comercial. Esses resultados sugerem que a maioria dos casos de *E. coli* piometra são causados por estirpes do filogrupo B2, e também indicam que a dieta pode influenciar a colonização intestinal por tais estirpes. A ocorrência simultânea de piometra em duas cadelas coabitantes foi investigada a fundo pela hipótese de transmissão entre esses animais. Tanto a tipagem de sequência multilocus do genoma inteiro quanto a análise de polimorfismo de nucleotídeo único apoiaram a hipótese de que os isolados do conteúdo uterino de ambos os animais e do reto de um eram clonais. Esse achado confirmou, pela primeira vez, a transmissão de *E. coli* associada à piometra entre dois animais. Dados clínicos, alterações histopatológicas e achados microbiológicos de cadelas com piometra (n=39) foram analisados para avaliar possíveis associações. Houve uma associação entre a detecção de *papC* em isolados de *E. coli* e maiores escores de necrose. Além disso, o escore de necrose associou-se positivamente ao tempo de internação, sendo que cada aumento de ponto no escore de necrose levou a mais dois dias de internação. Esses resultados sugerem que a *E. coli* positiva para *papC* desempenha um papel importante na gravidade da piometra em cães.

Palavras-chave: *Escherichia coli*, ExPEC, transmissão de piometra, patogenicidade bacteriana.

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LIST OF ABREVIATIONS

°C	degree Celsius
µm	micrometer
ALP	alkaline phosphatase
AMC	amoxicillin/clavulanic acid
AMP	ampicillin
bp	base pair
BUN	blood urea nitrogen
CC	clonal complex
CEH	cystic endometrial hyperplasia
CIP	ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CTF	ceftiofur
D	dog
DOX	doxycycline
ENO	enrofloxacin
EnPEC	endometrial pathogenic *Escherichia coli*.
ERIC-PCR	enterobacterial repetitive intergenic consensus - polymerase chain reaction
ExPEC	extraintestinal pathogenic *Escherichia coli*
GEN	gentamicin
goeBURST	global optimal - based upon related sequence type
h	hours
HE	hematoxylin and eosin
HV	veterinary hospital
Hz	Hertz
kbp	kilobase pair

kv	kilovolt
m/z	mass-to-charge ratio
MALDI-ToF	matrix-assisted laser desorption/ionization-time of flight
Max	maximum
MC	MacConkey agar
mg/dL	milligrams per deciliter
mg/mL	milligrams per milliliters
MH	Mueller Hinton agar
million/mm^2	millions of cells per cubic millimeter
Min	minimum
MLST	multilocus sequence typing
mm^2	cubic millimeter
MS	mass spectrometry
n	Number
NCBI	National Center for Biotechnology Information
NEO	neomycin
OHE	ovariohysterectomy
OT	oxytetracycline
p	*p* - value
P	uterine content
PCR	polymerase chain reaction
PEH	pseudoplacentacional endometrial hyperplasia
REP-PCR	random repetitive extragenic palindromic - polymerase chain reaction
RMBD	raw meat-based diet
RV	reference values
S	rectal swab
SLV	single locus variance

SNP	single-nucleotide polymorphism
ST	sequencing type
SUT	trimethoprim/sulfamethoxazole
U/L	units per liter
UFMG	Universidade Federal de Minas Gerais
wgMLST	whole-genome multilocus sequence typing
WGS	whole-genome sequencing
α	alpha

SUMMARY

1. INTRODUCTION

Pyometra is the most common reproductive disease in dogs, affecting an average of 25% of non-spayed bitches (Hagman, 2023). The disease is characterized by a bacterial infection in the uterus with local and systemic clinical manifestations ranging from purulent vulvar discharge to peritonitis, sepsis and dysfunction of various organs (Fieni et al., 2014; Henriques et al., 2014; Henriques et al., 2016). Studies suggest some factors that predispose the occurrence of pyometra, such as age over seven years and use of steroid hormones to prevent reproduction (Hagman et al., 2011; Jitpean et al., 2012). In addition, some breeds seem more predisposed, such as Boxer, Cocker Spaniel, Collie, Golden Retriever, Labrador, Pinscher, Rottweiler, Saint Bernard, Schnauzer and Chow Chow (Rautela and Katiyar, 2019). In these breeds, the involvement affects up to 50% of females (Hagman et al., 2011). Despite its great clinical infection, the pathogenesis of pyometra is still poorly understood, but it is believed that there is involvement of hormonal factors that apparently favor adhesion, colonization and bacterial growth in the organ (Johnson et al., 2001; Coggan et al., 2008; Ghanbarpour and Akhtardanesh, 2012). Thus, bacteria from the intestinal microbiota ascend to the uterus, causing the disease (Siqueira et al., 2009; Henriques et al., 2014; Hagman, 2023).

Among the bacteria that cause pyometra, *Escherichia coli* stands out as the most frequently isolated pathogen, being reported in up to 90% of cases (Hagman, 2023). Interestingly, such isolates are phylogenetically and epidemiologically distinct from strains commonly found as commensals of the intestine or causing diarrhea and other gastrointestinal disorders (Tenaillon et al., 2010; Abdallah et al., 2011; Coura et al., 2018). *E. coli* isolates are also commonly divided into three main categories composed of commensal strains, intestinal pathogenic (or diarrheagenic) strains, and extraintestinal pathogenic strains (Russo and Johnson, 2000; Tenaillon et al., 2010; Clermont et al., 2013). These groups differ due to several characteristics, emphasizing the presence of specific virulence factors that lead to the classification into pathotype (Johnson and Stell, 2000; Abdallah et al., 2011; Coura et al., 2018). Extraintestinal pathogenic *E. coli* (ExPEC) is the most common pathotype in several extraintestinal infections in animals and humans, causing meningitis, pyelonephritis, cystitis and septicemia, in addition to canine pyometra (Russo and Johnson, 2000; Siqueira et al., 2009; Salipante et al., 2015). ExPEC strains isolated from uterine contents of affected dogs commonly present virulence factors such

as adhesins, toxins, iron acquisition systems and serum resistance (Johnson and Stell, 2000; Siqueira et al., 2009), which may confer a selective advantage over commensal strains (Salipante et al., 2015). Studies also suggest that such factors play a key role in the development of pyometra (Chen et al., 2003; Mateus et al., 2013; Henriques et al., 2014).

Although ExPEC isolates are phylogenetically and epidemiologically distinct from more common commensal strains or even those that cause diarrhea (Abdallah et al., 2011), these isolates can efficiently colonize the intestinal tract (Johnson and Russo, 2002; Dale and Woodford, 2015). Therefore, it is believed that the ExPEC involved in canine pyometra originate in the intestine of the affected animal itself (Hagman, 2023). This hypothesis was reinforced by studies evaluating isolates from the uterine contents of infected bitches and from the feces of bitches not affected by the disease (Johnson et al., 2001; Chen et al., 2003; Siqueira et al., 2009; Mateus et al., 2013). Though, no studies comparing *E. coli* strains from the intestinal microbiota of dogs with pyometra caused by this agent with strains isolated from dogs with pyometra by other bacteria. The identification and comparison of these isolates would allow understanding the determining characteristics, the main mechanisms involved in the establishment of extraintestinal infections.

Although the hypothesis of intestinal tract ascension of *E. coli* strains is currently the most accepted in the pathogenesis of canine pyometra, there are no studies evaluating the influence of canine diet on the specific colonization of ExPEC strains. Previous works have suggested that dogs fed raw-meat based diets are known to increase the shedding of some *E. coli* pathotypes, suggesting that this group would be more predisposed to opportunistic infections in general (Kim et al., 2017; Davies et al., 2019; Ramos et al., 2022). On the other hand, specific virulence factors related to ExPEC were not investigated in these works. Therefore, evaluating the frequency of ExPEC in dogs fed raw-meat based diets and comparing them to commercial dog food may shed light on whether this new modality may increase or decrease the risk of extraintestinal infections associated with *E. coli.*

Currently, canine pyometra is considered a non-contagious bacterial disease (Chen et al., 2003; Hagman, 2022). So far, the possibility of transmission between individuals is not discussed and there are no studies evaluating this hypothesis. However, two cohabiting dogs were

diagnosed with pyometra within a week interval, raising the hypothesis of transmission. For a better evaluation of this hypothesis, it is necessary to compare uterine isolates and intestinal contents to assess similarity using molecular techniques. If confirmed, transmission between animals would have a great impact, especially in situations where a greater number of females live in the same place, such as kennels.

Some studies have associated certain clinical signs with the progression of the disease. It is known that fever and hypothermia are associated with a greater risk of developing peritonitis and death, while moderate to severe general depression and pale mucous membranes are associated with a longer hospitalization (Pretzer, 2008; Dąbrowski et al., 2013; Enginler et al., 2014; Jitpean et al., 2014). However, there are no studies evaluating the influence of certain pathogens on the severity of pyometra and it is still unknown whether the bacterial species involved in the infection can have an influence in the prognosis. Even for *E. coli*, the most commonly pathogen found in pyometra, there are no studies assessing whether the various virulence factors of ExPEC interfere with the individual prognosis. Therefore, the present study proposes an association between bacterial pathogenicity, endometrial histological changes and clinical prognosis in canine pyometra, data that can be of great help to the clinician in choosing the most appropriate treatment.

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2. OBJECTIVES

The aim of this study were: (1) to characterize *E. coli* isolates from uterine contents and feces of bitches affected by pyometra and feces from healthy dogs consuming two different diets; (2) determine the genetic similarity of *E. coli* isolates from the uterine contents and feces of two cohabiting bitches that were diagnosed with pyometra in the same period; (3) assess whether there is an association between bacterial pathogenicity, endometrial histological changes and clinical prognosis in canine pyometra.

3. CHAPTER 1. CANINE PYOMETRA: A SCOPING REVIEW OF CURRENT ADVANCES

ABSTRACT

Pyometra, characterized by the accumulation of purulent exudate in the uterus, is the most prevalent reproductive disease in canines. While the disease often begins with mild local symptoms, it can escalate to peritonitis, sepsis, and multi-organ dysfunction, thereby posing a significant threat to life. Despite the high incidence and recognized significance of canine pyometra, gaps persist in our understanding of its epidemiology, etiology, and pathogenesis. Recent studies have, however, broadened our comprehension of this disease, shedding light on potential new infection sources, etiologies, and the application of clinical predictive biomarkers and new protocols for therapy. This study aimed to review the current understanding of canine pyometra, with particular emphasis on the latest research concerning its etiology and epidemiology. Furthermore, it addressed key research questions and proposed directions for future investigations into various facets of canine pyometra.

Keywords: reproductive; *Escherichia coli*; uterine

3.1. Introduction

Pyometra is characterized by the accumulation of purulent exudate in the uterine lumen and it´s the most prevalent reproductive disease in canines (Hagman, 2018). It typically develops during the luteal phase, with *Escherichia coli* being the most frequently isolated bacteria (Kassé et al., 2016; Castillo et al., 2018; Rainey et al., 2018). Other commonly reported microorganisms include *Staphylococcus pseudintermedius* and *Streptococcus canis*. Recent studies, however, have suggested the potential involvement of less common pathogens, including *Brucella abortus*, *Corynebacterium* spp., and possibly *Porphyromonas* spp. (Wareth et al., 2017; Zheng et al., 2023).

Canine pyometra typically begins with subtle clinical signs such as polydipsia, polyuria, and vaginal discharge. Without timely treatment, it can progress to peritonitis, sepsis, and dysfunction of multiple organs (Jitpean et al., 2017; Hagman, 2022). Consequently, it is regarded as a life-threatening infection (Agostinho et al., 2014; Fieni et al., 2014; Jitpean et al., 2014b).

Despite the prevalent occurrence and recognized significance of canine pyometra, our understanding of its epidemiology, etiology, and pathogenesis remains incomplete. Recent studies have broadened our knowledge of this disease, identifying potential new infection sources, causes, and biomarkers that could aid in predicting its prognosis and severity. Consequently, this review aimed to consolidate the current knowledge on canine pyometra, with particular emphasis on the latest research concerning its etiology and epidemiology.

3.2. Epidemiology and risk factors

Pyometra, a bacterial infection in the uterus, is the most prevalent reproductive disease in dogs, impacting up to 25% of non-castrated females (Hagman, 2018). This disease is characterized by a bacterial infection in the uterus that results in local and systemic clinical signs (Chen et al., 2003; Fieni et al., 2014; Rautela and Katiyar, 2019). Although pyometra can occur in dogs ranging from 3 months to 20 years old, it predominantly affects middle-aged to older dogs (Figure 1), with a median diagnosis age of nine years (Martins et al., 2015; Lansubsakul et al., 2022; Xavier et al., 2023). The higher incidence of pyometra in middle-aged to older dogs is

thought to be associated with repeated estrous cycles. During diestrus, progesterone enhances the secretory activity of the endometrial glands, promotes endometrial proliferation, diminishes myometrium contractility, and induces cervix closure (Pretzer, 2008). Additionally, diestrus also reduces local leukocyte responses and uterine resistance to bacterial infection (Wijewardana et al., 2015; Hagman, 2018). These effects, which accumulate after repeated estrous cycles, escalate the risk of pyometra with each cycle (Pretzer, 2008; Martins et al., 2015; Sachan et al., 2019).

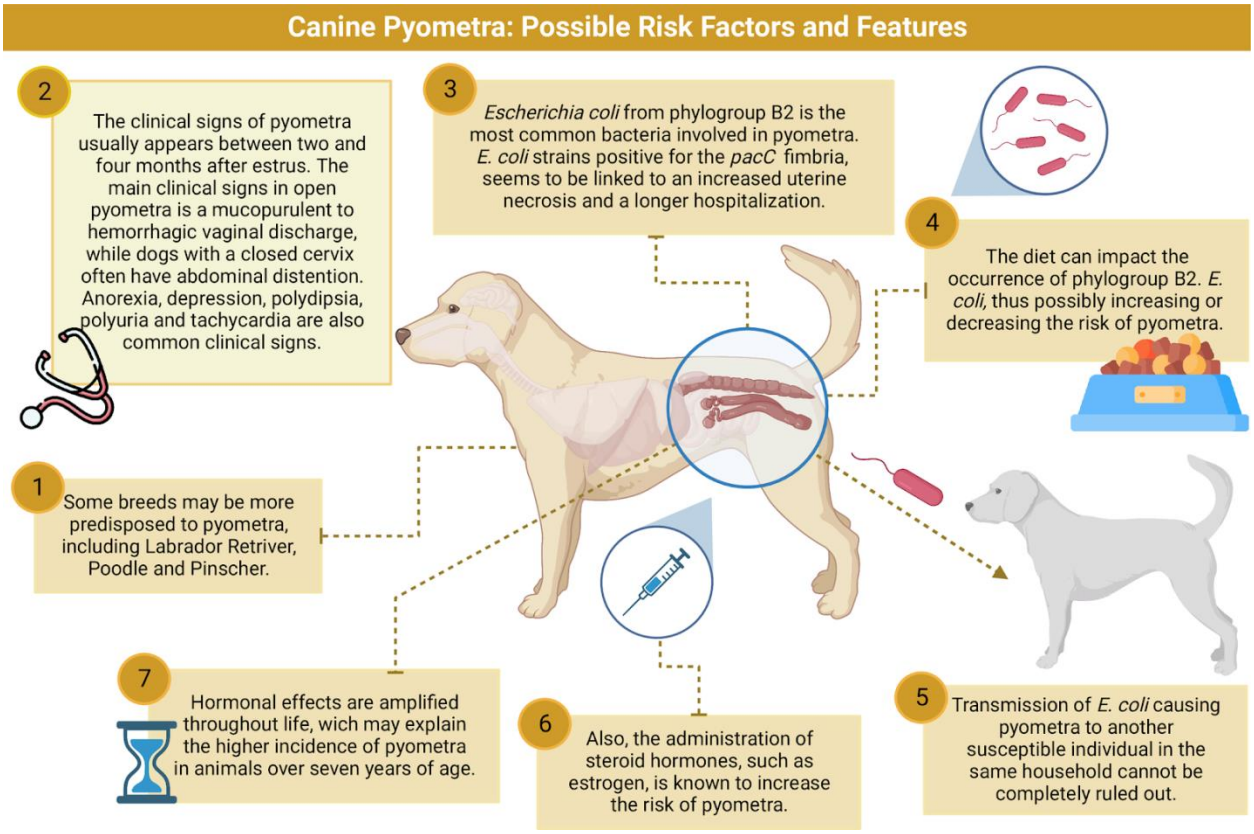

Figure 1. Infographic summarizing the possible risk factors and features of canine pyometra. Data from references (Smith, 2006; Whitehead, 2008; Martins et al., 2015; Wareth et al., 2017; Rautela and Katiyar, 2019; Sachan et al., 2019; Sala et al., 2021; Lansubsakul et al., 2022; Xavier et al., 2022b; Xavier et al., 2022a; Hagman, 2023; Zheng et al., 2023). Created using BioRender® (https://www.biorender.com/).

Some studies suggest that certain breeds may be more susceptible to pyometra (Table 1) (Younis et al., 2014; Antonov et al., 2015; Martins et al., 2015; Rautela and Katiyar, 2019). However, the prevalence of pyometra appears to fluctuate in studies con-ducted across various countries and the hypothesis of breed predisposition is just speculative. Recent research involving Golden Retriever has identified a potential correlation between pyometra and specific changes in the ABCC4 gene located on chromosome 22 (Arendt et al., 2021; Hagman, 2023). This discovery introduces, for the first time, a potential explanation for the increased incidence of pyometra in a particular breed. Despite this finding, there remains no definitive evidence of breed predisposition to pyometra, and the reasons for its higher prevalence in some breeds largely remain a mystery.

Table 1. Reported frequency of dog breeds affected by pyometra.

Breeds	Frequency (%)
Labrador Retriever	8-38
Poodle	10-33
Mixed-breed	27-30
Yorkshire Terrier	6-13
Pinscher	8-11
Golden Retriever	1-8
Rottweiler	1-8
Chow Chow	1-2
Others[1]	<1

[1]Other breeds include the American Pit Bull Terrier, Border Collie, German Shepherd, Lhasa Apso, Maltese, Pekingese, and Shih Tzu. Data from references (Sethi et al., 2020; Anjos et al., 2021; Chouksey et al., 2022; Hagman, 2023; Xavier et al., 2023).

The administration of drugs used for reproductive control, such as progestogens or estrogen compounds, is a recognized predisposing factor for canine pyometra (Dow, 1959; Smith, 2006; Whitehead, 2008; Sala et al., 2021). These drugs, which suppress the sexual receptivity phase in bitches, have been linked to an increased risk of pyometra and other conditions, including fetal maceration, endometrial and mammary tumors, and insulin resistance (Berky and Townsend, 1993; Niskanen and Thrusfield, 1998; Sala et al., 2021). Hormonal effects, which intensify over time, may account for the higher incidence pyometra in animals over seven years of age (Lansubsakul et al., 2022; Hagman, 2023).

3.3. Etiopathogenesis

Despite the high incidence of canine pyometra, its pathogenesis remains inadequately understood. It is evident, however, that the pathogenesis is multifactorial, involving bacterial infection, hormonal changes (or a favorable endocrine environment), genetic predisposition, and preexisting uterine lesions (Santana and Santos, 2021). During the luteal phase of the estrous cycle (diestrus), progesterone stimulates the proliferation and secretion of endometrial glands. Moreover, progesterone inhibits myometrial contraction and weakens the uterine immune response, thereby promoting bacterial colonization (Fieni et al., 2014; Prapaiwan et al., 2017; Rautela and Katiyar, 2019). Early studies on the pathogenesis of canine pyometra established a connection between hormonal stimulation and the occurrence of pyometra. At that time, cystic endometrial hyperplasia was considered a predisposing endometrial lesion leading to pyometra under experimental conditions (Dow, 1959). However, it was later discovered that, in addition to cystic endometrial hyperplasia, bitches in diestrus often develop another type of proliferative change in the endometrium. This change is characterized by endometrial hyperplasia with glandular cystic changes and decidual changes affecting the superficial endometrial epithelium, termed “pseudoplacentational endometrial hyperplasia” (Figure 2A and 2B) (Schlafer and Gifford, 2008). A recent study showed that in naturally occurring canine pyometra, pseudoplacentational endometrial hyperplasia is significantly associated with pyometra, whereas cystic endometrial hyperplasia is not (Santana et al., 2020). Notably, despite this significant association, a cause-and-effect relationship between pseudoplacentational endometrial hyperplasia and pyometra is yet to be established (Santana et al., 2020; Hagman, 2023). These recent findings (Santana et al., 2020) suggest that the traditional terminology of the “cystic endometrial hyperplasia-pyometra complex” is outdated (Santana and Santos, 2021). However, this should not be misinterpreted as diminishing the importance of endometrial hyperplastic changes in the pathogenesis of canine pyometra.

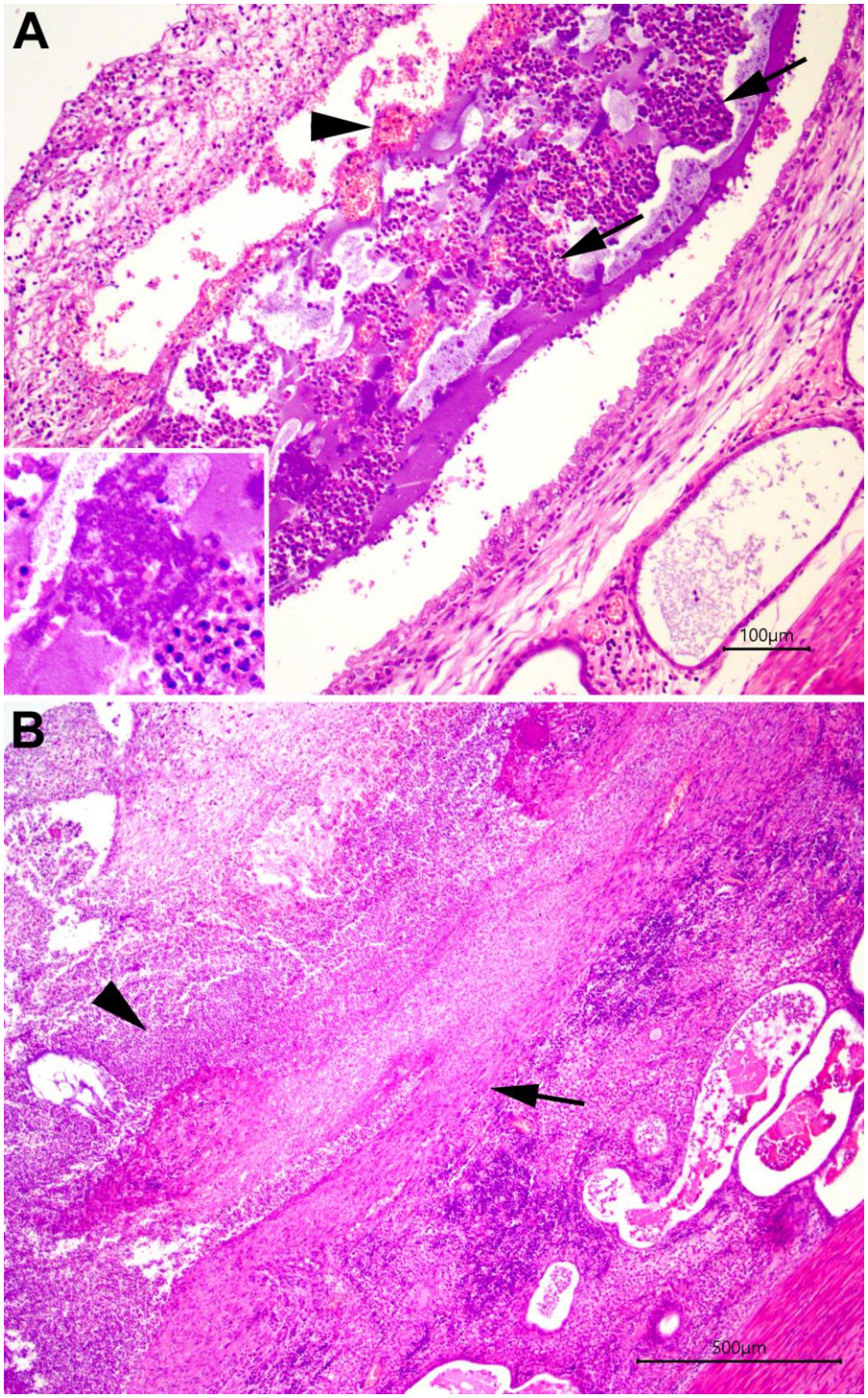

Figure 2. Uterus from a female dog with pyometra. (A) Endometrium with diffuse severe neutrophilic inflammatory infiltrate (arrows), with hemorrhage (arrowhead), fibrin and intraluminal bacterial aggregates (inset), and a columnar and vacuolated endometrial superficial

epithelium (decidual reaction) and ectasia of endometrial glands in a case of pseudoplacentacional endometrial hyperplasia. HE; bar = 100 μm. (B) endometrium with necrosis and superficial epithelial loss (arrow), with a diffuse severe neutrophilic inflammatory (arrowhead) infiltrate and mild hemorrhage. Endometrium with diffuse severe interstitial lymphoplasmacytic inflammation and marked glandular ectasia. HE; bar = 500 μm.

A broad spectrum of bacteria can contribute to pyometra in dogs (Young et al., 2017; Zheng et al., 2023). *E. coli* is among the most prevalent microorganisms, implicated in up to 90% of canine pyometra cases (Table 2). This gram-negative facultative anaerobic bacterium is also the primary pathogen in uterine infections across various species, including humans (McCain et al., 2009; Ikeda et al., 2013; Rainey et al., 2018). As *E. coli* is a component of the gut microbiota, it is postulated that this microorganism can ascend from the rectum to the uterus, thereby causing the disease. This theory is substantiated by studies demonstrating that the *E. coli* strains responsible for pyometra are often indistinguishable from those colonizing the gastrointestinal tract of the same dog (Wadås et al., 1996; Agostinho et al., 2014; Xavier et al., 2022b). Intriguingly, most dogs with pyometra are gut-colonized specifically by *E. coli* from phylogroup B2 (Xavier et al., 2022b), the same phylogroup frequently isolated from the uterine content of affected animals (Mateus et al., 2013; Henriques et al., 2014; Xavier et al., 2022b). Conversely, healthy dogs are more commonly gut-colonized by other phylogroups, including B1 (Mateus et al., 2013; Coura et al., 2018; Xavier et al., 2022b). This observation has led to the hypothesis that colonization by certain *E. coli* strains may elevate the risk of pyometra. In this context, a recent study demonstrated that diet can influence the colonization rate by *E. coli* from phylogroup B2 in the gut, suggesting that certain diets may indirectly heighten the risk of pyometra. If this hypothesis is further validated, strategies to alter or modulate the microbiota could provide an additional means to prevent or reduce the risk of pyometra (Xavier et al., 2022b).

In addition to phylogroup studies, researchers have examined the presence of virulence factors in *E. coli* isolated from canine pyometra. Some suggest that the possession of a specific combination of virulence genes may determine the severity of pyometra in bitches (Henriques et al., 2014; Lopes et al., 2021). Among these virulence factors, the gene encoding type P fimbriae (*papC*) has recently gathered significant attention. Firstly, the prevalence of this gene is often

higher in *E. coli* isolates from dogs with pyometra (ranging between 36.5 and 44.1%) compared to strains from the gut of healthy dogs (ranging between 18.2 and 29.2%) (Siqueira et al., 2009; Xavier et al., 2022b). Secondly, experimental studies have shown that this fimbria plays a crucial role in the adhesion and colonization of *E. coli* in the canine endometrium (Krekeler et al., 2013). A recent study also revealed a higher degree of uterine necrosis in dogs with pyometra caused by *E. coli papC*-positive strains. Interestingly, the degree of necrosis was positively correlated with the duration of hospitalization, suggesting a potential link between this fimbria and disease severity (Xavier et al., 2023). Another study proposed that uterine *E. coli* infection could alter the expression of sex hormone receptors in the uterus of bitches, thereby enhancing the hormonal factors that promote bacterial growth (Qian et al., 2020). Collectively, these studies strongly suggest that certain *E. coli* strains, possessing specific virulence traits, may be more likely to cause canine pyometra by facilitating tissue colonization and even modifying the uterine environment to favor infection. Further research is required to better understand the influence of gut microbiota and diet on the colonization by pyometra-causing *E. coli*.

Table 2. Most common bacterial species isolated from the uterus of bitches with pyometra.

Organism	Frequency (%)
Escherichia coli	28-90
Staphylococcus sp.	2-42
Klebsiella pneumoniae	2-33
Streptococcus sp.	4-25
Proteus mirabilis	1-17
Pseudomonas aeruginosa	1-16
Enterobacter sp.	1-11
Enterococcus sp.	<1-3
No growth	10-26

Data from references (Sethi et al., 2020; Anjos et al., 2021; Chouksey et al., 2022; Hagman, 2023; Xavier et al., 2023).

In addition to *E. coli*, other members of the Enterobacteriaceae family, such as *Klebsiella pneumoniae* and *Proteus mirabilis*, are frequently implicated in pyometra. Bacteria from the *Streptococcus*, *Staphylococcus*, and *Enterococcus* genera are also noteworthy (Table 2). Studies have demonstrated that, similar to *E. coli*, *K. pneumoniae*, *S. pseudintermedius*, *S. canis*, and *E.*

faecalis strains isolated from dogs with pyometra differ from most commensal strains. They express virulence factors such as adhesins, toxins, iron acquisition mechanisms, and mechanisms for evading the host immune system. These factors facilitate colonization and sustain the infection in the canine uterus (Hassan et al., 2003; Siqueira et al., 2009; Bachman et al., 2011; Gulhan et al., 2015; Pitchenin et al., 2017).

Studies have intriguingly reported that no microorganism is isolated in up to 25% of pyometra cases (Yoon et al., 2017; Hagman, 2022; Xavier et al., 2022b). Several hypotheses have been proposed to explain this phenomenon, including the host immune system's elimination of the pathogen, the use of antimicrobials during the preoperative period, the low sensitivity of culture methods, and the existence of microorganisms that do not grow in the standard culture media used for routine diagnosis (Yoon et al., 2017). This last hypothesis has been reinforced by studies that have identified the presence of some uncommon microorganisms causing pyometra, such as *Mycoplasma* spp., *Nocardia* spp., *Corynebacterium* spp., *Moraxella* spp., *Clostridium perfringens*, *Porphyromonas* spp., and *Brucella abortus* (Wareth et al., 2017; Hagman, 2023; Zheng et al., 2023). While the infection in most cases likely ascends from the gastrointestinal tract, the detection of certain specific bacteria, including *Brucella abortus*, suggests that other infection routes, such as hematogenous, are also possible (Wadås et al., 1996; Hagman and Kühn, 2002; Agostinho et al., 2014). Notably, *Porphyromonas* sp. has recently been confirmed as a cause of pyometra, leading to the hypothesis that bacteria typically found in the oral cavity can cause pyometra. Interestingly, *Porphyromonas* sp. is a well-established cause of reproductive diseases in humans, as well as endocarditis, lung, liver, and kidney infections, which it can spread through the bloodstream (hematogenously) (Hardham et al., 2005; Shub et al., 2006; Hashimoto et al., 2015; John et al., 2016; Ludovichetti et al., 2021).

3.4. Clinical presentation

Pyometra typically manifests local and systemic clinical signs (Figure 3), generally appearing between two and four months post-estrus (Agostinho et al., 2014; Fieni et al., 2014; Müştak et al., 2015). The most prevalent clinical symptom in dogs with open pyometra is the presence of a vaginal discharge that ranges from mucopurulent to hemorrhagic (Figure 4)

(Pretzer, 2008). Conversely, dogs with a closed cervix often exhibit abdominal distention owing to the lack of uterine content drainage (Figure 5) (Jitpean et al., 2017).

Clinical findings in pyometra cases can vary, but they commonly include inappetence/anorexia, depression/lethargy, polydipsia, polyuria, tachycardia, and tachypnea (Pretzer, 2008; Jitpean et al., 2014b; Hagman, 2022). Pyometra is a life-threatening condition because of the potential for complications such as uterine rupture, nephropathy, peritonitis, endotoxemia, and particularly sepsis (Maddens et al., 2010; Rautela and Katiyar, 2019; Santana et al., 2020).

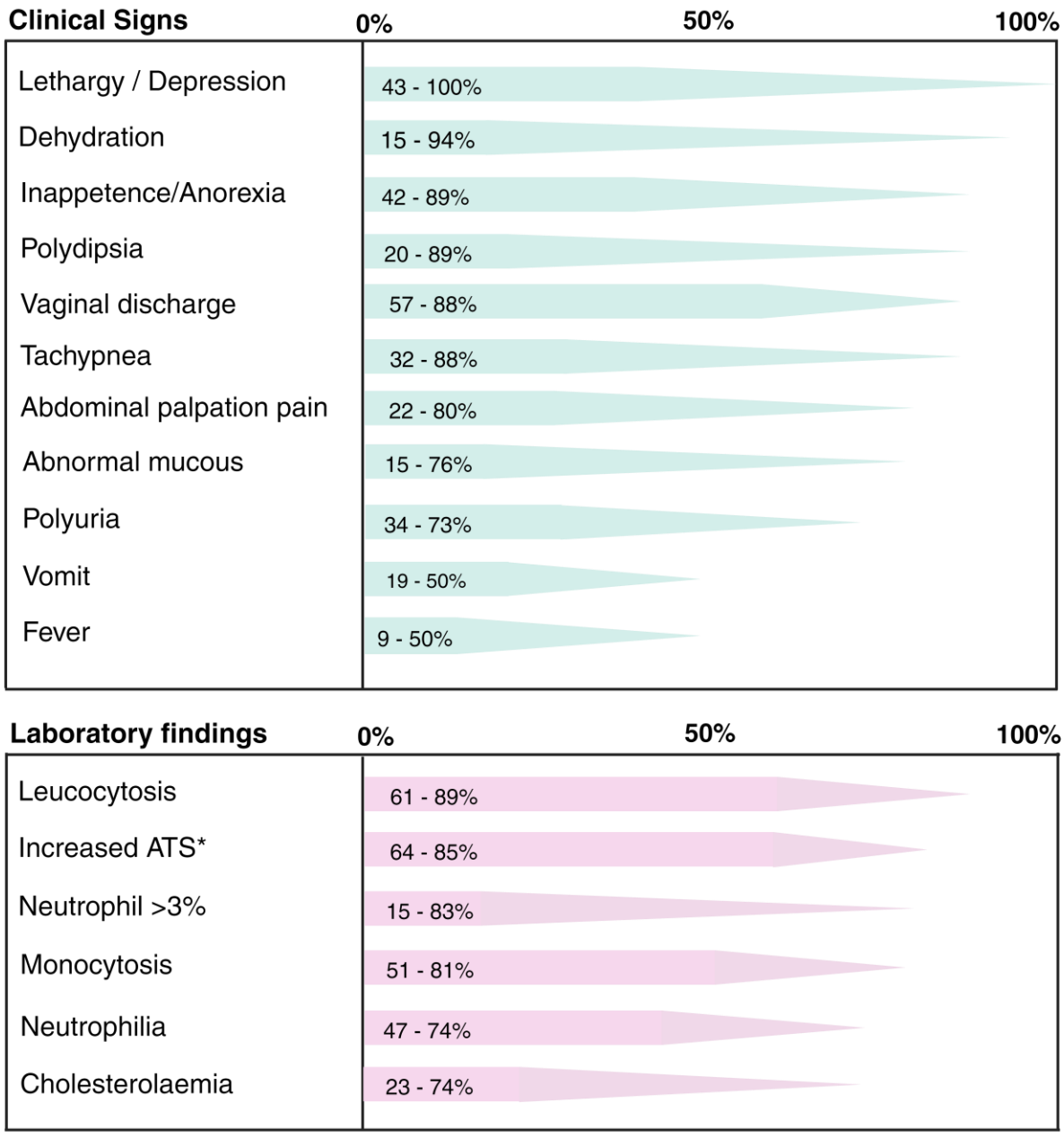

Figure 3. Main clinical signs and laboratory findings in bitches with pyometra, according to previous reports (Kaymaz et al., 1999; Jitpean et al., 2014b; Jitpean et al., 2017; Pailler et al., 2022; Talukdar et al., 2022; Xavier et al., 2022a; Hagman, 2023; Peixoto et al., 2023). *AST - Aspartate aminotransferase.

Figure 4. Purulent vaginal discharge in a bitch with open cervix pyometra.

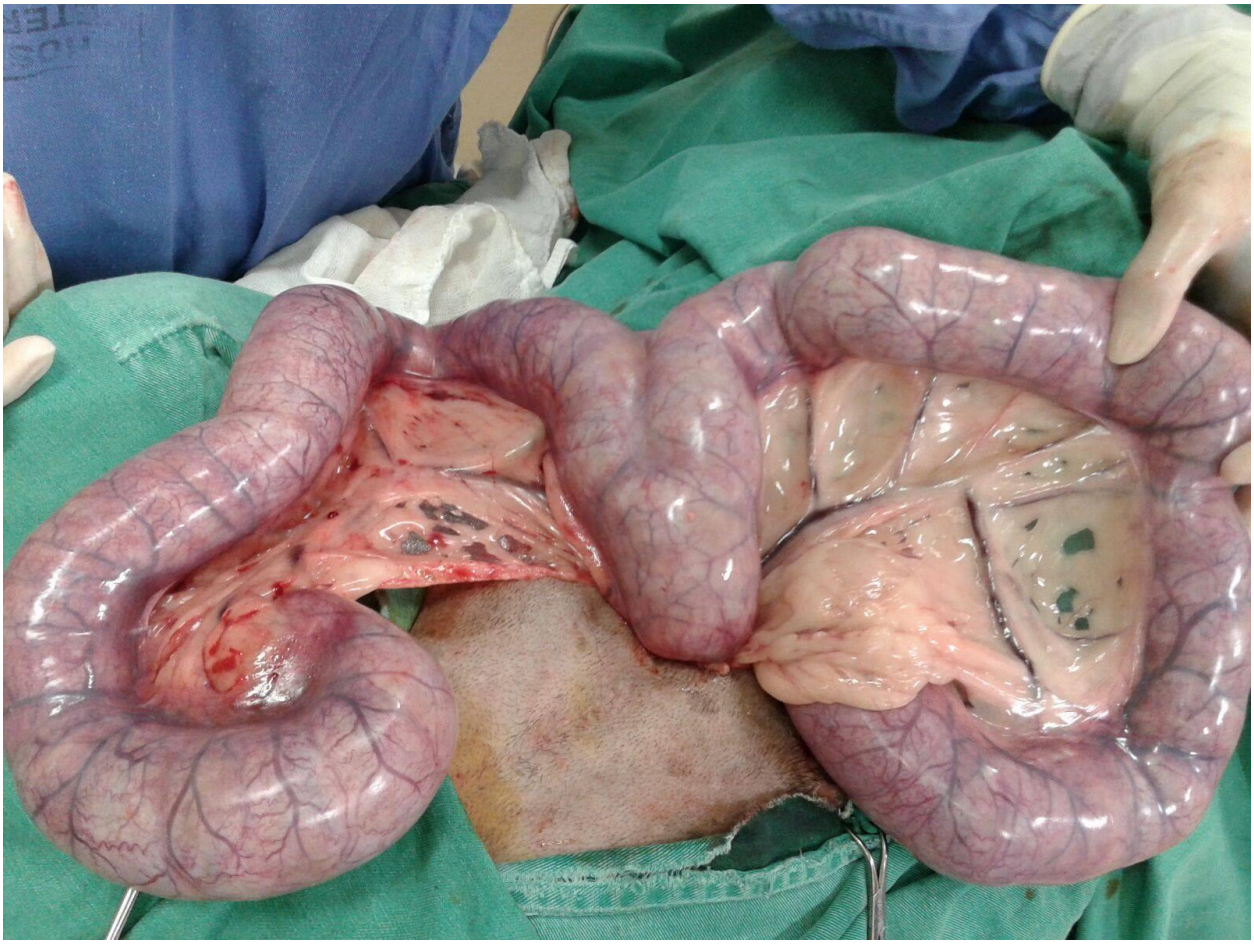

Figure 5. Intraoperative image of an enlarged, pus-filled uterus in a bitch (mixed-breed dog) with pyometra.

Notably, fever and hypothermia have been identified as factors increasing the risk of peritonitis development. Concurrently, moderate to severe general depression and pale mucous membranes are linked to extended hospitalization periods (Jitpean et al., 2014b). Furthermore, animals with closed pyometra exhibit a more severe condition and an elevated risk of sepsis (Jitpean et al., 2014a; Jitpean et al., 2017).

3.5. Diagnosis

The clinical diagnosis of the disease is often facilitated in cases of open pyometra. However, in the absence of vaginal discharge, diagnosis can be significantly more challenging owing to the variability of other clinical signs (Hagman, 2022). Typically, diagnosis relies on patient history, clinical signs, and imaging tests such as abdominal radiography and ultrasound. Additional tests, including blood count, leukogram, and liver function evaluation, can also provide valuable information (Figure 3) (Henriques et al., 2014; Jitpean et al., 2014b; Rautela and Katiyar, 2019). Frequently observed in affected animals are leukocytosis and anemia, along with signs of azotemia. This is because renal dysfunction can result from endotoxemia, glomerular dysfunction, renal tubular damage, and a decreased response to the antidiuretic hormone (Hagman, 2022).

Ultrasonography proves beneficial in identifying intrauterine fluid, even when the uterine diameter falls within the normal range (Figure 6). Additionally, it offers the advantage of revealing further pathological alterations in the tissue and ovaries, such as ovarian cysts or cystic endometrial hyperplasia (Bigliardi et al., 2004; Hagman, 2022).

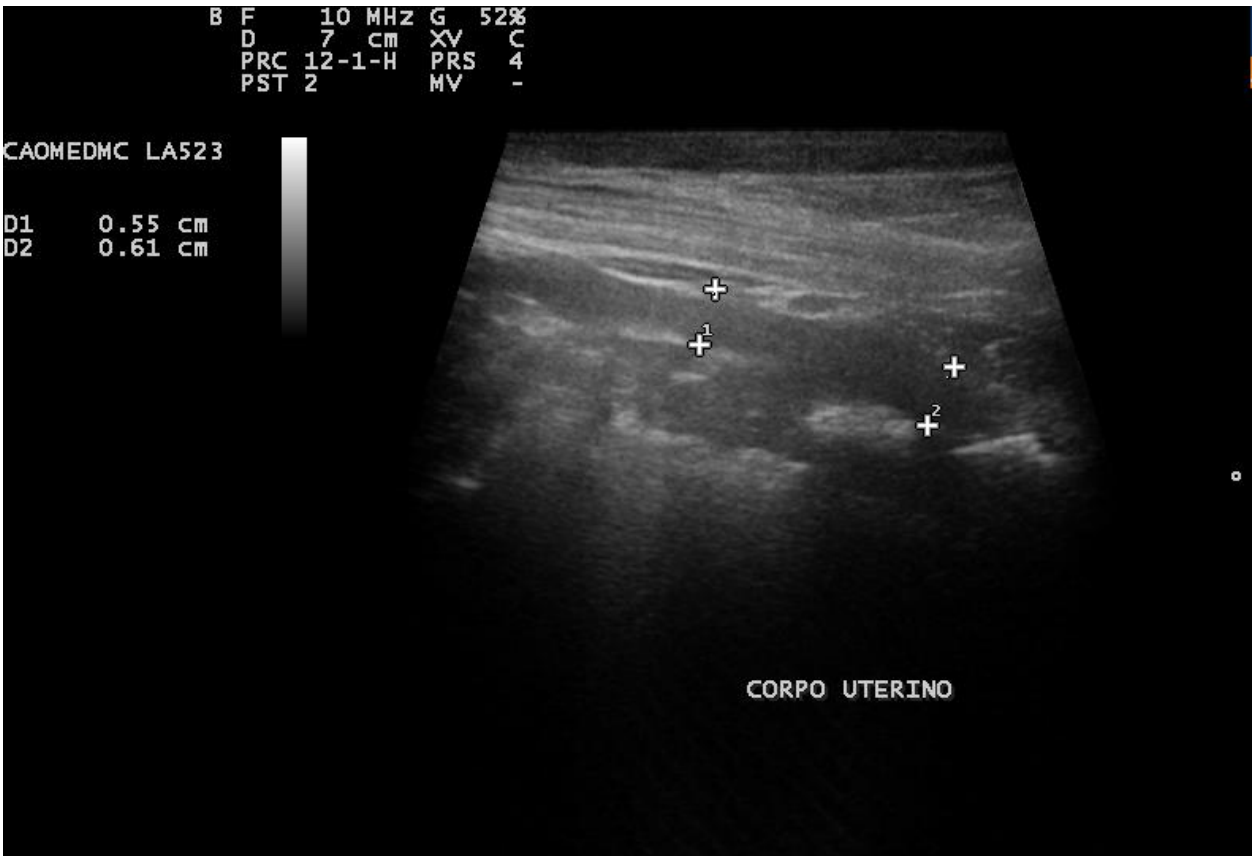

Figure 6. Abdominal ultrasound image of the uterus of a Pinscher. An enlarged left uterine horn measuring approximately 4.26 cm in diameter in transverse plane is noted (cursors), with hypoechogenic content, related to pyometra.

While not commonly requested, additional complementary examinations may prove beneficial. These include histopathological analysis of the uterus following ovariohysterectomy, and microbiological culture of uterine content. These tests can confirm a diagnosis of pyometra, identify the bacteria associated with the infection, and facilitate antimicrobial susceptibility testing of the isolate (Hagman, 2023).

3.6. Treatment

Pyometra is a medical emergency that requires prompt attention and ovariohyster-ectomy (OHE) is still the preferred treatment. Typically, the patient's overall clinical condition reverts to normal within two weeks once the infection source is eliminated (Agostinho et al., 2014; Jitpean et al., 2017). However, the procedure's primary drawback is permanent sterility, which is particularly significant if the owner has breeding interest in the animal (Fieni et al., 2014). Complications associated with OHE include hemorrhage, accidental ureteral ligation, estrogen-responsive urinary incontinence, ovarian remnant syndrome, and stump pyometra (Howe, 2006; Ball et al., 2010). Stump pyometra may develop post-OHE if a section of the uterine horns or body remains, and the animal exhibits elevated progesterone levels and/or an ovarian remnant (Howe, 2006; Ball et al., 2010; Ehrhardt et al., 2023). The clinical manifestation, diagnosis, and treatment of stump pyometra are similar to those of pyometra, except for the history of a prior OHE (Hagman, 2023).

While antibiotic therapy is frequently incorporated into the standard treatment protocol for pyometra, some researchers propose that perioperative antimicrobials should be reserved for animals exhibiting moderate to severe depression, thereby minimizing unnecessary antimicrobial usage (Axnér et al., 2016; Turkki et al., 2023). In such instances, the initial selection of antimicrobial should be effective against *E. coli*, the most prevalent bacteria implicated, and ideally, adjusted based on culture and antibiogram results to a personalized narrow-spectrum alternative for each patient, thereby mitigating the risk of selecting multidrug-resistant bacteria (Ghanbarpour and Akhtardanesh, 2012; Lopes et al., 2021). However, it is worth noting that the majority of veterinarians seldom, if ever, request these culture tests (Lavin and Maki, 2023).

Fluoroquinolones, such as enrofloxacin, and amoxicillin/clavulanate are the primary and secondary recommendations for pyometra treatment according to the Antibiotic Use Guidelines for Companion Animal Practice (Table 3) (Jessen et al., 2019). Conversely, the Finnish and Swedish guidelines propose sulfadoxine-trimethoprim and ampicillin as the preferred choices, respectively (Axnér et al., 2016; EVIRA, 2016). Research from various countries indicates that most anti-microbials, including those recommended by these guidelines, are largely effective against isolates from canine pyometra. Other effective compounds include cephalothin, streptomycin, and gentamicin (Hagman and Greko, 2005; Inoue et al., 2013; Agostinho et al., 2014; Rocha et al., 2021; Lansubsakul et al., 2022). A recent retrospective review corroborated

these findings by demonstrating that ampicillin or amoxicillin are effective antimicrobials for cases requiring antibiotic treatment, particularly in dogs exhibiting moderate to severe general demeanor depression (Turkki et al., 2023).

Table 3. Antimicrobials recommended for antibiotic therapy in bitches with pyometra.

Drugs	Dosage	Reference
Sulfadoxine-trimethoprim	15mg/kg/q 12h	(Axnér et al., 2016)
Ampicillin	10-20mg/kg/q 6-8h	(EVIRA, 2016)
Enrofloxacin	2.5-5.0 mg/kg/q 12h	(Jessen et al., 2019)
Amoxicillin/clavulanate	10-20mg/kg/q 12h	

Solely pharmacological treatment has been exclusively utilized in certain scenarios, such as with young breeders or when anesthesia and surgery are not currently feasible (Jitpean et al., 2017; Sperling et al., 2018; Melandri et al., 2019). Importantly, the animal should have open cervix pyometra and no signs of ovarian cystis (Hagman, 2022). The goal during the pharmacologic management of pyometra is to actively expel the purulent contents from the uterus and inhibit bacterial growth, promoting uterine healing. Consequently, the protocols typically involve the simultaneous administration of steroids, antiprogestative, and antimicrobials (Table 4). Aglepristone, a progesterone receptor blocker, and cloprostenol, a synthetic prostaglandin F2∝ (PGF2α) analogue (Fieni et al., 2014; Ros et al., 2014; Contri et al., 2015).

Table 4. Examples of protocols used for pharmaceutical treatment of open cervix pyometra in bitches.

Drugs	Dosage	Frequency	Reference
Aglepristone	10mg/kg q 24h	Three doses. Days 1, 2 and 7 or 2, 7 and 14 or 1, 2 and 7 Four doses. Days 1, 3, 6 and 9	(Trasch et al., 2003; Jurka et al., 2010; Contri et al., 2015)
Aglepristone Cloprostenol	10mg/kg q 24h 1 µg/kg SC q 24	Days 1, 3, 8 and 15 Days 3 and 8	(Gobello et al., 2003)

In addition to these drugs, antimicrobial therapy (preferably based on sensitivity tests) and supportive treatment are essential. It is important to note that these protocols are not recommended for dogs exhibiting certain clinical signs such as fever, hypothermia, liver and/or

kidney failure, or suspected peritonitis. Bitches subjected to non-surgical treatment need to be close monitored considering the risk of drug side effects, but also due to the risk of rapid general health deterioration, mostly linked to sepsis and endotoxemia. Also, the owner should be aware that recurrence is possible and that pregnancy rates and litter sizes following pharmacological treatment can be lower than those in healthy animals. This is attributed to the belief that endometrial lesions in cases of canine pyometra impair the dog's ability to conceive or sustain a pregnancy (Fieni, 2006; England et al., 2007; Fieni et al., 2014; Hagman, 2018).

3.7. Predictive markers

Research has attempted to link prognosis with the identification of certain biomarkers (Ahn et al., 2021). Factors such as leukopenia, inappetence, azotemia, reduced packed cell volume, and dehydration have been correlated with extended hospitalization following OHE (Ahn et al., 2021; Pailler et al., 2022; Hagman, 2023). Additionally, leukopenia has been connected with the incidence of peritonitis (Jitpean et al., 2014b; Hagman, 2023).

C-reactive protein (CRP) is arguably the most extensively researched biomarker in dogs with pyometra. Current knowledge suggests that CRP levels decrease gradually following OHE, with sustained or increased concentrations potentially indicating complications (Hagman, 2017; Ahn et al., 2021; Hagman, 2023). Similarly, serum amyloid A, the hormone procalcitonin, and cell-free DNA exhibit the same pattern (Hagman, 2017; Ahn et al., 2021; Matur et al., 2021; Hagman, 2023). CRP levels are also elevated in dogs with pyometra and sepsis compared to those with mucometra (Soler et al., 2021). Consequently, some researchers have proposed that CRP could serve as a marker for severe cases or to distinguish pyometra from mucometra (Pretzer, 2008; Dąbrowski et al., 2013; Enginler et al., 2014). The level of CRP has been directly linked to the length of the postoperative period (Fransson et al., 2007), suggesting its potential as a valuable prognostic tool. Other studies have proposed that serum amyloid A and cell-free DNA could be used for sepsis screening, while interleukin-6 and high mobility group Box 1 might be useful for therapeutic monitoring of sepsis (Fransson et al., 2007; Jitpean et al., 2014a; Ahn et al., 2021). Regrettably, these biomarkers are not yet routinely used in most veterinary hospitals. Conversely, some parameters commonly included in routine testing, such as serum creatinine and

urinary gamma-glutamyl transpeptidase, have not proven clinically useful in determining the severity of pyometra or renal injury in affected dogs (Braz, 2021).

3.8. Prevention

Elective OHE ("spay") serves as the primary method for pyometra prevention. However, sterilization can lead to adverse side effects, including surgical and anesthetic complications, a heightened occurrence of certain musculoskeletal and endocrinological disorders, obesity, and urinary incontinence in bitches (Root Kustritz, 2012). It is crucial to meticulously evaluate the advantages and disadvantages of such a procedure in each case, considering the breed of the animal (Howe, 2006; Kutzler, 2020; Kutzler, 2023).

The potential for pathogenic *E. coli* to ascend from the intestinal tract to the uterus has been documented (Xavier et al., 2022b; Hagman, 2023). This finding suggests that future research could explore how various diets influence intestinal colonization by bacteria that cause pyometra, potentially leading to preventative measures for this condition in dogs. A recent case study reported the transmission of pyometra between two Chow Chow dogs. Although the mechanisms are not fully understood, it is suggested that isolating healthy cohabiting animals from dogs with purulent vaginal discharge (indicative of open cervix pyometra) could potentially prevent disease transmission (Xavier et al., 2022a).

3.9. Future perspectives

Canine pyometra, a potentially lethal and commonly occurring reproductive disease in bitches, is known to be influenced by pre-existing uterine lesions and hormonal and bacterial factors. However, its pathogenesis remains largely elusive. Our understanding of the etiological factors involved in *E. coli*-induced pyometra, as well as the role of other pathogens, is continually evolving. Future research may elucidate the influence of diet and intestinal microbiota on pyometra risk, potentially aiding in the development of more effective prevention protocols for this enduringly prevalent disease. Although challenging, it is necessary to elucidate if some

breeds are indeed at a higher risk of pyometra. If confirmed, understanding the mechanisms behind this susceptibility can help developing novel strategies to prevent and reduce pyometra incidence.

Recent works also described the involvement of less common pathogens in pyometra, raising the hypothesis that other infection routes, including hematogenous, might be more common than previously anticipated. In this context, the hypothesis of microorganisms from the oral microbiota causing pyometra should be further explored.

3.10. Conclusions

Despite the known significance of canine pyometra, our understanding of its epidemiology and etiopathogenesis is still incomplete. Pyometra is known as a multifactorial disease that occurs commonly during the diestrus. Some established risk factors include age and the use of drugs for reproductive control, such as progestogens or estrogen compounds. It is also likely that some breeds are more predisposed to the infection, although this hypothesis was not fully proved yet. Bitches with cystic endometrial hyperplasia or pseudoplacentational endometrial hyperplasia seem also to be at higher risk of developing the infection. Ovariohysterectomy (OHE) is still the preferred treatment for canine pyometra but pharmacological treatment, commonly with aglepristone, is also possible in some specific cases.

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3.12. References

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4. CHAPTER 2. CHARACTERIZATION OF *Escherichia coli* IN DOGS WITH PYOMETRA AND THE INFLUENCE OF DIET ON THE INTESTINAL COLONIZATION OF EXTRAINTESTINAL PATHOGENIC *E. coli* (EXPEC)

ABSTRACT

Despite its high frequency and clinical relevance, the pathogenesis of canine pyometra remains poorly understood. The most accepted hypothesis is that bacteria involved ascend from the intestinal tract, causing the uterine infection. Extraintestinal pathogenic *Escherichia coli* (ExPEC) is the most frequent pathogen in canine pyometra, accounting for 57–100% of cases. The aim of the present study was to determine the frequency of phylogenetic groups and virulence factors in *E. coli* strains isolated from the uterine and rectal swabs of bitches with pyometra (n = 72) and from rectal swabs from healthy bitches fed commercial dry feed (n = 53) or a raw meat-based diet (RMBD; n = 38). A total of 512 strains of *E. coli* were isolated and divided into five categories according to the origin of the sample: 120 isolates from the uterine content of dogs with *E. coli* pyometra, 102 from the feces of bitches with *E. coli* pyometra, 75 from the feces of bitches without *E. coli* pyometra, 130 feces samples from healthy dogs fed commercial feed, and 85 feces samples from healthy dogs fed a raw meat-based diet. *E. coli* strains belonging to the B2 phylogroup and positive for virulence factor genes associated with adhesion (fimbriae type P [*papC*]) and production of toxins (α-hemolysin [*hlyA*] and uropathogenic specific protein [*usp*]) predominated in the uterine content and rectal swabs of bitches with *E. coli* pyometra. Interestingly, a lower growth rate of *E. coli* from the B2 phylogroup was observed in dogs fed a RMBD than in those fed commercial dry feed. The present study suggests that intestinal colonization by certain types of *E. coli* could be a risk factor for the occurrence of *E. coli* pyometra in bitches and that diet can influence intestinal colonization by such strains.

Keywords: EnPEC; UPEC; RMDB; uterus; uterine; microbiota.

4.1. Introduction

Pyometra is the most frequently occurring reproductive disease in bitches, affecting up to 25% of uncastrated females (Hagman, 2018). The disease is characterized by bacterial infection of the uterus with local and systemic clinical manifestations that can lead to death (Fieni, 2006; Jitpean et al., 2014; Müştak et al., 2015). However, despite its relevance, the pathogenesis of this disease remains poorly understood. It is believed that bacterial species causing pyometra ascend from the intestinal tract of females, causing infections (Chen et al., 2003; Müştak et al., 2015; Hagman, 2022).

Extraintestinal pathogenic *Escherichia coli* (ExPEC) is the most common pathogen involved in canine pyometra and has been reported in 57–100% of cases (Chen et al., 2003; Lopes et al., 2020; Hagman, 2022). These isolates are phylogenetically and epidemiologically distinct from *E. coli* strains commonly found in intestinal commensals that cause diarrhea and other gastrointestinal disorders (Tenaillon et al., 2010; Abdallah et al., 2011; Coura et al., 2021). In canine pyometra, *E. coli* strains found in uterine contents are commonly associated with phylogroup B2 and less frequently with phylogroup D (Liu et al., 2008; Mateus et al., 2013; Lopes et al., 2020). In contrast, commensal intestinal strains of *E. coli* in dogs are mostly classified into phylogenetic groups B1 and A (Müştak et al., 2015; Liu et al., 2017; Coura et al., 2021). In addition, *E. coli* recovered from pyometra have specific virulence factors, such as adhesins, toxins, iron acquisition systems, and protectins (Maluta et al., 2014; Henriques et al., 2016; Lopes et al., 2021), which are commonly classified as endometrial pathogenic *E. coli* (EnPEC), a subgroup of the ExPEC pathotype. These virulence factors may confer a selective advantage over commensal strains (Salipante et al., 2015), playing a key role in the development of canine pyometra (Russo and Johnson, 2000; Henriques et al., 2014; Henriques et al., 2016) as well as in other extraintestinal infections in humans and animals (Russo and Johnson, 2000; Siqueira et al., 2009; Salipante et al., 2015).

Although the intestinal ascension of *E. coli* strains is currently the most accepted hypothesis in the pathogenesis of canine pyometra (Lopes et al., 2021; Hagman, 2022), no studies have evaluated the influence of the dog diet on the specific colonization of ExPEC strains. In the last decade, an increasing number of owners have been feeding their dogs and cats a raw meat-

based diet (RMBD), instead of regular commercial dry feed (Viegas et al., 2020). Dogs fed an RMBD shed an increased amount of some pathogens in their feces, including *Salmonella* spp. and diarrheagenic *E. coli* (Kim et al., 2017; Davies et al., 2019; Viegas et al., 2020). However, specific virulence factors related to extraintestinal infections have not yet been investigated. The aim of this study was to determine the prevalence of phylogroups and virulence factors in *E. coli* isolates obtained from the uterine contents and feces of bitches with pyometra infection. In addition, we compared these *E. coli* isolates with those obtained from the feces of healthy dogs fed commercial dry feed or an RMBD to evaluate the possible influence of diet on colonization by *E. coli* strains.

4.2. Materials and methods

4.2.1. Sampling

Three groups of bitches were sampled in the present study: dogs with pyometra (uterine and rectal swabs), healthy dogs fed commercial dry feed (rectal swab), and healthy dogs fed a RMBD (rectal swab). A total of 72 bitches with pyometra who underwent ovariohysterectomy (OHE) surgery at the Veterinary Hospital of the Universidade Federal de Minas Gerais (VH-UFMG) between January 2017 and December 2020 were included. Immediately following surgery, aspiration puncture of the uterine contents was performed and a swab was introduced into the rectal ampulla of the bitches. The samples were refrigerated at 4 °C until processing for a maximum of 24 h. Rectal swabs from 91 healthy dogs were included, of which 53 were fed commercial dry feed, and 38 were fed a RMBD from a previously published study (Viegas et al., 2020). The samples were kept in a cooler with ice packs and transported for processing within a maximum of 24 h. This study was approved by the Ethical Committee on Animal Use of UFMG (protocol nº. 51/2015).

4.2.2. Isolation and identification of *E. coli*

The uterine contents were plated on Mueller Hinton (MH) agar (Kasvi, Maharashtra, India) supplemented with equine blood (5%) and MacConkey (MC) agar (Difco, Franklin Lakes, NJ, USA), and the plates were incubated at 37 °C for 48 h under aerobiosis and anaerobiosis. Plating of rectal swab samples from bitches subjected to OHE and healthy dogs was performed on MC agar and incubated at 37 °C for 48 h under aerobiosis. For each clinical specimen, up to three lactose-fermenting colonies were subjected to species-specific polymerase chain reaction (PCR) to identify *E. coli* (McDaniels et al., 1996). Strains not identified as *E. coli* was identified by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-ToF MS; Bruker Daltonics, Billerica, USA). A cutoff log score of 2 was used to validate the identification at the species level, as recommended by the manufacturer.

4.2.3. Characterization of *E. coli*

E. coli strains were subjected to PCR to determine phylogroups (A, B1, B2, C, D, E, F, or clade I) (Clermont et al., 2013), and identify virulence genes corresponding to the ExPEC pathotype, namely, fimbriae type I (*fimH*), fimbriae type I central region (*focG*), fimbriae type P (*papC* and *papG*, allele II and III), fimbriae type S (*sfaS*), cytotoxic necrotizing factor type 1 (*cnf1*), uropathogenic specific protein (*usp*), α-hemolysin (*hlyA*), aerobactin (*iutA*), and serum resistance (*traT*) (Johnson and Stell, 2000; Siqueira et al., 2009).

4.2.4. Statistical analysis

The results were analyzed using EngineRoom software (MoreSteam, 2009). To analyze the association between *E. coli* phylogroups, virulence factors, and categorical variables related to the group origin of the samples (uterine content of bitches with *E. coli* pyometra, rectal swabs of bitches with *E. coli* pyometra, or without *E. coli* pyometra, and healthy dogs fed commercial dry feed or a RMBD) a multiple proportion comparison test was conducted. This test is based on the chi-square distribution and the pooled estimate of the population proportion to estimate the standard error of the test statistic. If a significant difference was found in the overall test, the pairwise comparisons method with Marascuillo procedure was used to identify the specific pairs

of proportions which differ significantly. Statistical significance of the results was set at $p \leq 0.05$ for the analyzed characteristics (Marascuilo, 1966).

4.3. Results

4.3.1. *E. coli* isolation

A total of 40 (56%) of the 72 dogs tested positive for *E. coli* in the uterine content; up to three colonies were obtained from each, totaling 120 *E. coli* strains, while 21 (29%) had only other pathogens, and no bacterial growth was seen in 11 (15%) (Table 5).

Table 5. Bacterial species isolated from the uterus in bitches with pyometra.

Organism	Total Cases (%)
Escherichia coli	40 (56)
Staphylococcus sp.	6 (8)
Streptococcus sp.	6 (8)
Enterobacter sp.	2 (3)
Enterococcus sp.	2 (3)
Klebsiella pneumoniae	2 (3)
Proteus mirabilis	2 (3)
Pseudomonas aeruginosa	1 (1)
No growth	11 (15)

Up to three colonies of *E. coli* were obtained from rectal swabs of 59 bitches with pyometra, totaling 177 *E. coli* strains: 102 from dogs that tested positive for *E. coli* content (*E. coli* pyometra) and 75 from bitches that tested negative for *E. coli* (without *E. coli* pyometra) in the uterine contents.

From healthy bitches, at least one *E. coli* isolate was recovered from 91 dogs sampled, totaling 215 strains: 130 and 85 from dogs fed commercial feed or RMBD, respectively.

4.3.2. *E. coli* phylogroups

Phylogroup B2 was the most common *E. coli* phylogroup detected in the uterine contents of bitches infected with *E. coli* pyometra (85%) and also in the rectal swab isolates of bitches with *E. coli* pyometra (58.8%), whereas B1 was most frequent in the rectal swabs of bitches without *E. coli* pyometra (41.3%). Bitches with *E. coli* pyometra showed a higher frequency of phylogroup B2 in the rectal swab than females without *E. coli* pyometra ($p<0.05$). Phylogroup B2 was also the most frequent in *E. coli* isolates from rectal swabs of dogs fed commercial dry feed (34.6%), whereas B1 was the most common in dogs fed RMBD (34.1%). Dogs fed commercial dry feed showed a higher frequency of phylogroup B2 in rectal swabs than dogs fed RMBD ($p<0.05$) (Table 6).

Table 6. Number of isolates and frequency of *Escherichia coli* phylogroups identified in the uterine content, rectal swabs of bitches with pyometra and rectal swabs of healthy dogs.

	Bitches with Pyometra		**Healthy Dogs**		
	Uterine Content		**Rectal Swab**		
Phylogroup	***E. coli* Pyometra**	***E. coli* Pyometra**	**Non-*E. coli* Pyometra**	**Consume Commercial Dry Feed**	**Consume RMBD**
A	0	2 (1.9%)	5 (6.6%)	4 (3%)	6 (7%)
B1	3 (2.5%)	22 (21.5%)	31 (41.3%)	35 (26.9%)	29 (34.1%)
B2	102 (85%)	60 (58.8%)[a]	18 (24%)	45 (34.6%)[b]	8 (9.4%)
C	0	4 (3.9%)	0	16 (12.3%)	11 (12.9%)
D	0	0	2 (2.6%)	1 (0.7%)	0
E	6 (5%)	7 (6.8%)	6 (8%)	10 (7.6%)	20 (23.5%)
F	3 (2.5%)	3 (2.9%)	10 (13.3%)	11 (8.4%)	10 (11.7%)
E. clades—clade I	0	0	0	3 (2.3%)	0
Not classified	6 (5%)	4 (3.9%)	3 (4%)	5 (3.8%)	1 (1.1%)
Total	**120**	**102**	**75**	**130**	**85**

[a]Samples with statistical difference when comparing strains obtained from the rectal swab of bitches with *E. coli* pyometra and bitches with non-*E. coli* pyometra. [b]Samples with statistical difference when comparing strains obtained from the rectal swabs of dogs fed commercial dry food and dogs fed RMBD.

4.3.3. Frequency of virulence genes associated with the ExPEC pathotype

All the virulence genes tested were detected in *E. coli* isolates from all groups at different frequencies. Virulence genes associated with adhesion (*papC*) and toxin production (*hlyA* and *usp*) were more frequent in the rectal swabs of bitches with *E. coli* pyometra than in those without *E. coli* pyometra ($p<0.05$). In addition, two virulence genes associated with adhesion (*focG* and *sfaS*) were more frequent in isolates from dogs fed commercial dry feed than in those

from dogs fed RMBD ($p<0.05$). In contrast, the serum resistance gene (*traT*) was found at a higher frequency in isolates from dogs fed RMBD than in those from dogs fed commercial dry feed ($p<0.05$) (Table 7).

Table 7. Number of isolates and frequency of *Escherichia coli* virulence genes identified in the uterine content, rectal swabs of bitches with pyometra and rectal swabs of healthy dogs.

	Bitches with Pyometra		**Healthy Dogs**		
	Uterine Content		**Rectal Swab**		
Virulence Genes	***E. coli* Pyometra**	***E. coli* Pyometra**	**Non-*E. coli* Pyometra**	**Consume Commercial Dry Feed**	**Consume RMBD**
Adhesion					
fimH	120 (100%)	102 (100%)	75 (100%)	128 (98.4%)	83 (97.6%)
focG	66 (55%)	56 (54.9%)	25 (33.3%)	47 (36.2%)[b]	15 (17.6%)
papC	66 (55%)	45 (44.1%)[a]	16 (21.3%)	38 (29.2%)	14 (16.4%)
papG	58 (48.3%)	36 (35.2%)	13 (17.3%)	74 (56.9%)	37 (43.5%)
sfaS	27 (22.5%)	16 (15.6%)	4 (5.3%)	26 (20%)[b]	1 (1.1%)
Toxins					
cnf1	50 (41.6%)	33 (32.3%)	14 (18.6%)	20 (15.3%)	9 (10.5%)
hlyA	61 (50.8%)	40 (39.2%)[a]	13 (17.3%)	22 (16.9%)	10 (11.7%)
usp	48 (40%)	28 (27.4%)[a]	4 (5.3%)	16 (12.3%)	5 (5.8%)
Iron acquisition					
iutA	43 (35.8%)	39 (38.2%)	26 (34.6%)	103 (79.2%)	74 (87%)
Serum resistance					
traT	76 (63.3%)	57 (55.8%)	48 (64%)	91 (70%)	84 (98.8%)[b]
Total	**120**	**102**	**75**	**130**	**85**

[a]Samples with statistical difference when comparing strains obtained from the rectal swab of bitches with *E. coli* pyometra and bitches with non-*E. coli* pyometra. [b]Samples with statistical difference when comparing strains obtained from the rectal swabs of dogs that consume commercial dry food and dogs that consume RMBD.

4.4. Discussion

As expected, *E. coli* was isolated from most of the uterine contents of dogs with pyometra. This result is in accordance with previous studies showing that *E. coli* is the main bacterium involved in pyometra (Chen et al., 2003; Castillo et al., 2018; Rainey et al., 2018).

Differentiation into phylogenetic groups and the detection of virulence factors have been widely used in studies on *E. coli*, helping to elucidate the epidemiology of infections and the colonization dynamics of these bacteria (Johnson and Stell, 2000; Clermont et al., 2015; Müştak et al., 2015). Previous studies have demonstrated that ExPEC strains isolated from canine

pyometra tend to cluster mainly in phylogroup B2, whereas those isolated from the intestinal microbiota of healthy dogs cluster mainly in phylogenetic groups B1 and A (Schmidt et al., 2015; Liu et al., 2017; Coura et al., 2018). In the present study, phylogroup B2 was the most frequent in the uterine contents of bitches, with clinical cases of pyometra caused by *E. coli* (Figure 7), corresponding to 85% of the isolates. This frequency is similar to that found in previous studies on pyometra, suggesting a high capacity of phylogroup B2 strains to colonize the canine uterus (Mateus et al., 2013; Henriques et al., 2014; Lopes et al., 2021).

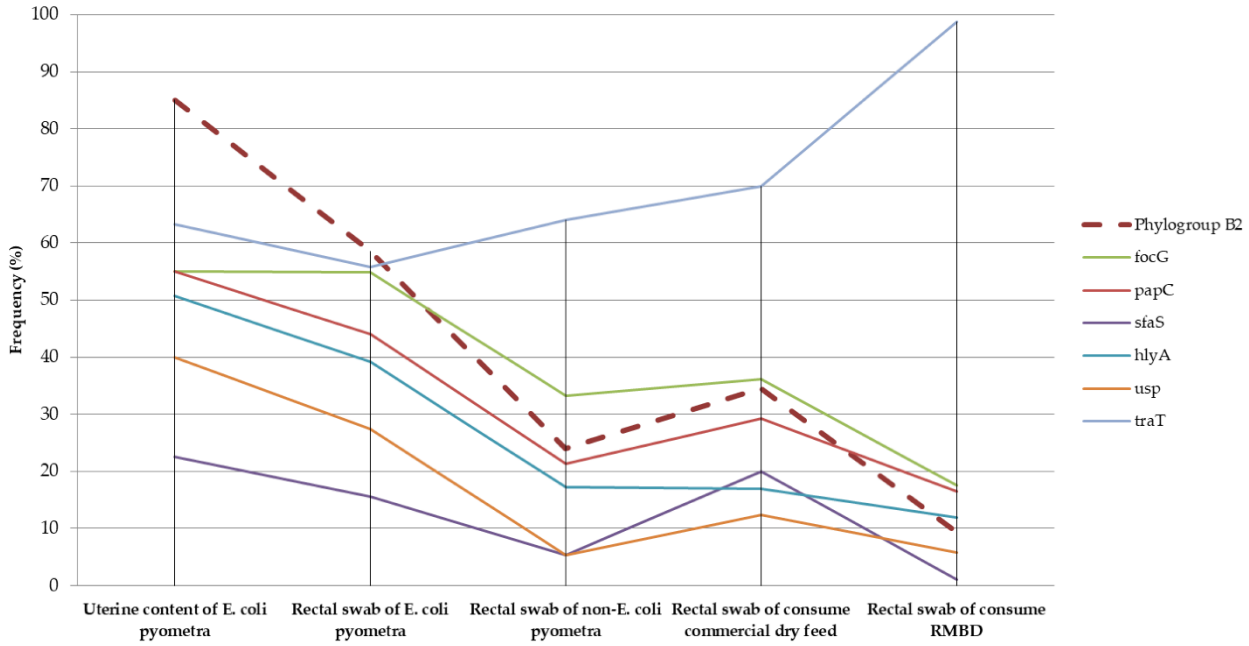

Figure 7. Frequency of the phylogroup B2 and the main virulence factors identified in *Escherichia coli* isolated from the uterine content, rectal swabs of bitches with pyometra and rectal swabs of healthy dogs fed commercial dry feed and raw meat-based diet (RMBD).

Although the pathogenesis of pyometra is poorly understood, previous studies have suggested that the intestine is the main source of *E. coli* strains that ascend into the uterus (Lopes et al., 2021; Hagman, 2022). This study reinforces this hypothesis, as bitches with *E. coli* pyometra were more likely to harbor *E. coli* strains from phylogroup B2 in the rectal swab when

compared to the group of bitches without *E. coli* pyometra. This finding indicates that intestinal colonization by *E. coli* from phylogroup B2 increases the risk of pyometra in bitches.

Another interesting aspect of ExPEC is the presence of certain virulence factors that enable infection at different locations (Müştak et al., 2015). Virulence factors that promote adhesion and colonization, especially fimbriae, are considered to be of great relevance for the establishment of *E. coli* infections in the canine uterus (Siqueira et al., 2009; Krekeler et al., 2012; Agostinho et al., 2014). Previous studies demonstrated that simple inactivation of some adhesins, such as type 1 (*fim*), P (*papGIII*), and S (*sfa/foc*) fimbriae, results in a considerable reduction in bacterial binding to cell lines of the canine endometrium, reinforcing the importance of these factors in the pathogenesis of the disease (Krekeler et al., 2013). In the present study, four adhesin-encoding virulence genes were found more frequently in *E. coli* samples obtained from the uterine contents, similar to the findings of previous studies (Siqueira et al., 2009; Mateus et al., 2013; Henriques et al., 2014). This finding reinforces the hypothesis that some adherent virulence factors are associated with pyometra caused by *E. coli* in bitches. It is noteworthy that the gene encoding type P fimbriae (*papC*), which is considered important for the adhesion and colonization of *E. coli* in the canine endometrium (Chen et al., 2003; Lopes et al., 2021), was found in 55% of the isolates from the uterine contents. This frequency is similar to that identified in other studies on canine pyometra isolates (Siqueira et al., 2009; Müştak et al., 2015). In addition, strains isolated from the rectal swabs of bitches with *E. coli* pyometra were more commonly positive for the type P fimbriae gene (*papC*) than strains isolated from the rectal swabs of dogs without *E. coli* pyometra. Notably, the frequency of *papC*-positive *E. coli* strains in dogs without *E. coli* pyometra was similar to that reported in a previous study on *E. coli* from rectal swabs of healthy dogs (Russo and Johnson, 2000).

Although *E. coli* is known to be the main bacterium involved in pyometra (Hagman, 2022), and recent studies have suggested that diet can influence *E. coli* colonization (Tenaillon et al., 2010; Wotzka et al., 2019; Kreuzer and Hardt, 2020), current studies have evaluated how different diets would affect the frequency of ExPEC in bitches. In the present study, the *papC* gene showed no statistical difference between the groups of healthy bitches under different types of feeding. In contrast, the genes encoding type 1 adhesin (*focG*) and S (*sfaS*) fimbriae were found less frequently in *E. coli* strains recovered from dogs fed RMBD. These adhesins are

considered important in the pathogenesis of canine pyometra (Ghanbarpour and Akhtardanesh, 2012; Krekeler et al., 2012; Mateus et al., 2013). However, it is important to note that the frequency of these two adhesin-encoding genes was similar in strains isolated from rectal swabs of dogs with or without *E. coli* pyometra, raising doubts regarding the role of these virulence factors in disease development.

Previous studies have indicated that ExPEC obtained from the uterine content of bitches with pyometra commonly expresses genes encoding toxins that may provide a selective advantage (Coggan et al., 2008; Lopes et al., 2021; Hagman, 2022). We observed that all toxin-coding virulence genes were found more frequently in *E. coli* samples obtained from the uterine content, which is in agreement with previous studies (Chen et al., 2003; Siqueira et al., 2009; Henriques et al., 2014), which reinforces the hypothesis that, in addition to adhesins, ExPEC toxin virulence factors are associated with the occurrence of *E. coli* pyometra. Among the *E. coli* isolates from rectal swabs, the α-hemolysin (*hlyA*) toxin, which is capable of lysing erythrocytes and leukocytes (Coggan et al., 2008; Henriques et al., 2014; Dale and Woodford, 2015), was found more frequently in strains isolated from bitches with *E. coli* pyometra than in strains isolated from rectal swabs of dogs without *E. coli* pyometra. Additionally, the uropathogenic specific protein (*usp*), which acts as a bacteriocin and assists in the migration of strains into the bloodstream (Siqueira et al., 2009; Agostinho et al., 2014; Etefia and Ben, 2020), was more frequent in strains isolated from the rectal swabs of bitches with *E. coli* pyometra than in strains isolated from dogs without *E. coli* pyometra.

ExPEC obtained from the uterine contents of bitches with pyometra is commonly positive for the aerobactin gene (*iutA*), a virulence factor responsible for iron acquisition (Dale and Woodford, 2015; Müştak et al., 2015), and for the serum resistance gene (*traT*), a virulence factor associated with the inhibition of the immune response of the host in cases of translocation of the pathogen into the bloodstream (Henriques et al., 2014; Dale and Woodford, 2015). In the present study, both virulence genes were detected in all groups, and the frequency was similar among *E. coli* strains obtained from uterine content and rectal swabs from bitches with *E. coli* and without *E. coli* pyometra. In contrast, *traT* was more frequently detected in *E. coli* strains from rectal swabs of dogs fed RMBD than in those fed commercial dry feed.

Research on phylogroups and virulence factors of *E. coli* from different origins has increased over the last few years, but many gaps remain, mostly regarding *E. coli* colonization and infection in dogs (Lopes et al., 2021; Hagman, 2022). In the present study, we demonstrated that, compared to dogs without *E. coli* pyometra, dogs with *E. coli* pyometra are more likely to be colonized by *E. coli* from phylogroup B2, which is positive for specific virulence genes, including type 1 adhesin (*papC*) and two toxins (*hlyA* and *usp*). These results suggest that colonization by these strains is a risk factor for canine pyometra caused by *E. coli*. Based on these results, we sampled two groups of healthy dogs under different diets to evaluate whether dietary habits altered the intestinal microbiota and further established *E. coli* in the B2 phylogroup. Our results suggest that dogs fed RMBD are less frequently colonized by *E. coli* strains from phylogroup B2, raising the hypothesis that diet can impact the frequency of phylogroup B2 *E. coli*, which is the main bacterium responsible for this disease (Chen et al., 2003; Hagman, 2017; Lopes et al., 2021).

Importantly, several studies have indicated public health risks associated with RMBD, such as greater fecal shedding of pathogenic and zoonotic microorganisms, which is a potential risk to animal and human health (Freeman et al., 2013; Viegas et al., 2020). Therefore, several health agencies have released statements that discourage the inclusion of raw or undercooked animal protein in dog diets (Freeman et al., 2013). We believe that the results of this study will motivate future evaluations of different diets for dogs that aim to reduce the colonization of ExPEC, however, this study should not be considered as a motivation for the adoption of RMDB, owing to the known risks of this practice.

4.5. Conclusion

The present study demonstrated the high frequency of *E. coli* strains belonging to phylogroup B2 and carrying virulence factors associated with ExPEC in isolates from the uterine contents of bitches with pyometra. In addition, this study found a higher frequency of these strains in the intestinal microbiota of bitches with *E. coli* pyometra than in bitches without *E. coli* pyometra, suggesting that intestinal colonization by these strains could be a risk factor for the occurrence of *E. coli* pyometra in dogs. Interestingly, when evaluating the intestinal microbiota

of dogs on different types of diets, the present study found a lower frequency of such strains in the intestinal microbiota of dogs subjected to a RMBD than in dogs who consumed commercial dry feed, suggesting that future studies on diet modulation affecting intestinal colonization could find mechanisms to prevent and control *E. coli* pyometra in dogs.

4.6. Acknowledgments

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4.7. References

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5. CHAPTER 3. TRANSMISSION OF *Escherichia coli* CAUSING PYOMETRA BETWEEN TWO BITCHES

ABSTRACT

Despite its clinical relevance, the pathogenesis of canine pyometra remains poorly understood. To date, it is recognized as a non-transmissible infectious disease. In this study, the simultaneous occurrence of pyometra and *Escherichia coli* in two cohabitant bitches underwent in-depth investigation due to the hypothesis of transmission between these animals. Two 5-year-old Chow Chow dogs (namely, dogs 23 and 24—D23 and D24) were referred to a veterinary hospital with suspected pyometra. Both animals showed prostration, anorexia, and purulent vulvar discharge over a 1-week period. After ovariohysterectomy, uterine tissue, uterine contents, and rectal swabs were collected for histopathological and microbiological analysis. Uterine histology demonstrated purulent material and multifocal necrosis with endometrial ulceration, and a morphological diagnosis of pyometra was confirmed. Furthermore, *E. coli* from the same phylogroup (B2) and positive for the same virulence factors with the same antimicrobial susceptibility profile was isolated from the uterine contents of both dogs and the rectum of D23. Conversely, the *E. coli* strains recovered from D24 differed in phylogroup (one isolate), virulence factors (all three isolates), and antimicrobial susceptibility (all three isolates). Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) suggested that all isolates from the uterine content of both dogs and the rectal swab of D23 were 100% the same, but different from all isolates in the rectal swab of D24. One isolate from the uterine content of each animal as well as rectal swabs were subjected to whole-genome sequencing (WGS). Both whole-genome multilocus sequence typing (wgMLST) and single-nucleotide polymorphism (SNP) analysis supported the hypothesis that the isolates from the uterine content of both animals and the rectal swab of D23 were clonal. Taken together, these clinical features, pathology, microbiology, and molecular findings suggest, to the best of our knowledge, the first transmission of *E. coli* associated with pyometra between two animals. These results could impact the management of sites where several females cohabit in the same local area such as kennels.

Keywords: ExPEC; EnPEC; UPEC.

5.1. Short Communication/Note

Pyometra is the most frequently observed reproductive disease in bitches, affecting up to 25% of unspayed females (Hagman, 2018). The disease is characterized by bacterial infection of the uterus with local and potentially fatal systemic clinical manifestations such as prostration, anorexia, purulent vulvar discharge, sepsis, and multi-organ dysfunction (Fieni, 2006; Jitpean et al., 2014; Müştak et al., 2015). Despite its relevance, the pathogenesis of this disease remains poorly understood. It is believed that bacterial species may cause pyometra to ascend from the host's intestinal tract, causing a non-transmissible opportunistic infection (Chen et al., 2003; Müştak et al., 2015; Hagman, 2022). In this study, we describe an in-depth investigation of the possible transmission of *Escherichia coli* associated with pyometra in two bitches.

A 5-year-old female Chow Chow (D23) was referred to the Veterinary Hospital of the Universidade Federal de Minas Gerais (VH-UFMG) with a purulent vulvar discharge. In addition to the vulvar discharge, the animal was hyperthermic (41 °C) and showed signs of prostration, anorexia, and diarrhea, suggestive of an open pyometra. The examinations also revealed anemia (hematocrit: 36%; RV: 37–55%), thrombocytopenia (platelets: 124,000/mm^2; RV: 175,000–500,000/mm^2, and azotemia (creatinine: 1.57 mg/dL; RV: 0.5–1.5 mg/dL). The animal underwent ovariohysterectomy (OHE) surgery. Just after the procedure, a sample from the uterine content and feces from the rectal ampulla of the dog were collected by needle aspiration and swab, respectively. The samples were refrigerated at 4 °C until processing for a maximum of 24 h. Samples from the uteri and ovaries were collected for histopathological analysis.

After 5 days, another female dog (D24) from the same litter and cohabiting with D23 was referred to VH-UFMG with similar symptoms including prostration, anorexia, and purulent vulvar discharge. These two dogs were the only animals in their household. Examination results indicated anemia (erythrocytes: 4.48 million/mm^2; RV: 5.5–8.5 million/mm^2 and hematocrit 25%), leukocytosis (leukocytes: 28,200 mm^2; RV: 6000–17,000 mm^2), thrombocytopenia (platelets: 90,000 mm^2), decreased blood urea nitrogen (BUN: 17.69 mg/dL; RV: 20–56 mg/dL), increased alkaline phosphatase (ALP: 157 U/L; RV: 40–156 U/L). This animal also underwent OHE surgery and again, uterine tissue, uterine content, and rectal swab samples were collected. After surgery, both animals were treated with amoxicillin/clavulanic acid and metronidazole.

Samples from the uteri and ovaries of both animals were fixed by immersion in 10% buffered formalin for 24 h, processed for paraffin embedding, and sectioned (3-µm thick), and stained with hematoxylin and eosin (HE) for histopathology. The uteri of both bitches were enlarged and filled with a significant amount of purulent brown material. Both animals were in diestrus, which was confirmed by the discovery of multiple corpora lutea. In addition, the ovaries of one bitch (D23) had neutrophilic arteritis and fibrinous thrombi, partially occluding the artery.

Microscopically, in both animals, the uterine lumen was filled with many neutrophils, fibrin, bacterial aggregates, and, in D23, also blood. There was a severe diffuse neutrophilic and lympho-histioplasmacytic endometrial inflammatory infiltrate, with marked neutrophilic exocytosis into the uterine lumen and endometrial glands. In D23, there was severe multifocal necrosis with endometrial ulceration extending to the superficial endometrial glands, with intense endometrial hemorrhage, fibrin deposition, and moderate fibroplasia (Figure 8); many other endometrial veins were filled with fibrin thrombi, which partially occluded the lumen. In D24, there was mild multifocal endometrial ulceration. These concurrent findings are highly indicative of the histopathological lesions observed in cases with severe pyometra (Schlafer and Gifford, 2008; Santana and Santos, 2021). Endometrial necrosis and ulceration may also be observed in pyometra cases; however, which determines the intensity of necrosis in each case is not well-defined (Santana and Santos, 2021). In D23, the remaining endometrial epithelial cells and in D24, epithelial cells of the luminal endometrial epithelium and superficial endometrial glands were columnar with finely vacuolated cytoplasm, morphologically indicative of decidual reaction. In both animals, endometrial glands were diffusely and markedly ecstatic with the accumulation of neutrophils and mucous (Santana and Santos, 2021) (Figure 8). In addition, a multifocal moderate histioplasmacytic and neutrophilic inflammatory infiltrate extended into the myometrium. Therefore, a morphological diagnosis of pseudoplacentational endometrial hyperplasia and pyometra was established in both bitches (Schlafer and Gifford, 2008). Interestingly, as observed in these bitches, a recent study described the high frequency of the association of pseudoplacentational endometrial hyperplasia with pyometra in bitches (Santana et al., 2020).

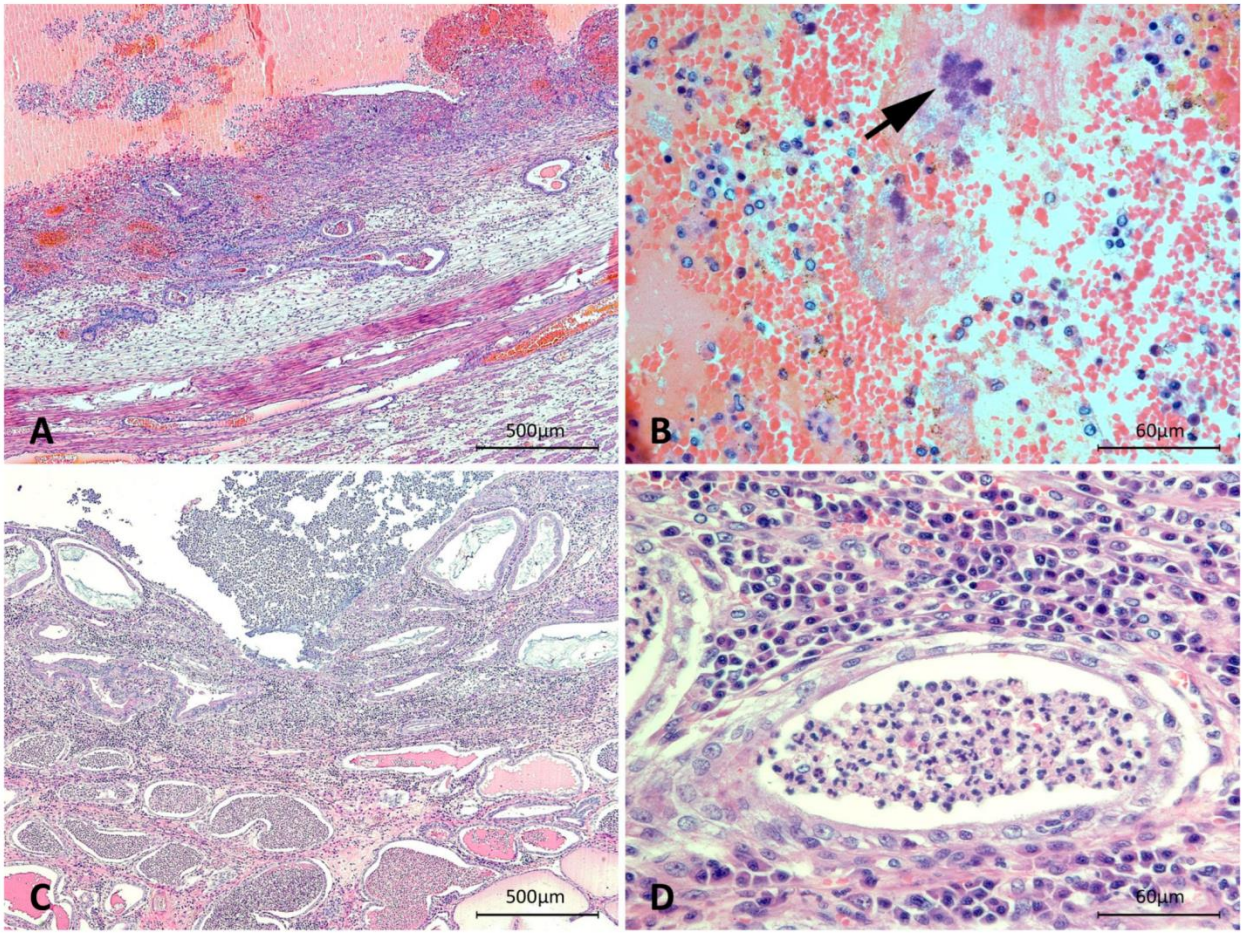

Figure 8. Uterine histopathology of dogs D23 and D24. (A) D23 - uterine lumen filled with large amounts of blood and increased cellularity in the endometrium, with severe necrosis, endometrial ulceration, and hemorrhage. Endometrial glands are markedly ecstatic. (B) D23 - higher magnification of A: hemorrhagic uterine luminal content, with neutrophils, plasma cells, fibrin, and bacterial aggregates (arrow). (C) D24 - severe diffuse endometritis, with endometrial glands markedly ecstatic with an accumulation of inflammatory cells and mucous, and superficial luminal and glandular epithelium composed of columnar and finely vacuolated cells. (D) D24 - higher magnification of C: severe diffuse interstitial lympho-histioplasmacytic infiltrate, and an endometrial gland filled with neutrophils. HE, scale bars = 500 µm (A,C), 60 µm (B,D).

The uterine contents and rectal swabs were plated on Mueller Hinton agar (Sparks, BD, USA) supplemented with 5% equine blood and in MacConkey agar (Kasvi, São José dos Pinhais,

Brazil), followed by aerobic and anaerobic incubation at 37 °C for 48 h. Three lactose-fermenting colonies from each sample (twelve isolates in total) were subjected to a species-specific polymerase chain reaction (PCR) to identify *E. coli* (McDaniels et al., 1996). Thus, the isolates were subjected to PCR to determine *E. coli* phylogroups (Clermont et al., 2013) and to detect the main virulence genes associated with the extraintestinal pathogenic *E. coli* (ExPEC) pathotype, namely, fimbriae type I (*fimH*), fimbriae type I central region (*focG*), fimbriae type P (*papC* and *papG* alleles II and III), fimbriae type S (*sfaS*), cytotoxic necrotizing factor type 1 (*cnf1*), α-hemolysin (*hlyA*), uropathogenic specific protein (*usp*), aerobactin (*iutA*), and serum resistance (*traT*) (Johnson and Stell, 2000; Siqueira et al., 2009). Additionally, antimicrobial susceptibility to amoxicillin/clavulanic acid, ampicillin, ceftiofur, ciprofloxacin, enrofloxacin, neomycin, gentamicin, doxycycline, oxytetracycline, and trimethoprim/sulfamethoxazole was assessed using the disk diffusion method and interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2020; CLSI, 2021).

All *E. coli* strains isolated from the uterine contents of both bitches were classified into the same phylogroup (B2) (Figure 9). This result was not surprising as *E. coli* is the most common bacterial organism found in a pyometra, and these isolates tend to cluster mainly in phylogroup B2 (Müştak et al., 2015; Lopes et al., 2021; Xavier et al., 2022). Furthermore, the uterine *E. coli* isolates also had the same ExPEC virulence factor-encoding genes (*fimH*, *focG*, *papC*, *papG*, *cnf1*, *hlyA*, and *usp*) (Figure 9), virulence factors also commonly associated with endometrial pathogenic *E. coli* (Lopes et al., 2020; Lopes et al., 2021). These virulence factors, mainly the fimbriae-encoding genes (*fimH*, *focG*, *papC* and *papG*), are of great relevance for the establishment of *E. coli* infections in the canine uterus (Siqueira et al., 2009; Agostinho et al., 2014; Lopes et al., 2021). Additionally, *E. coli* strains from the uterine contents had no antimicrobial resistance (Figure 9).

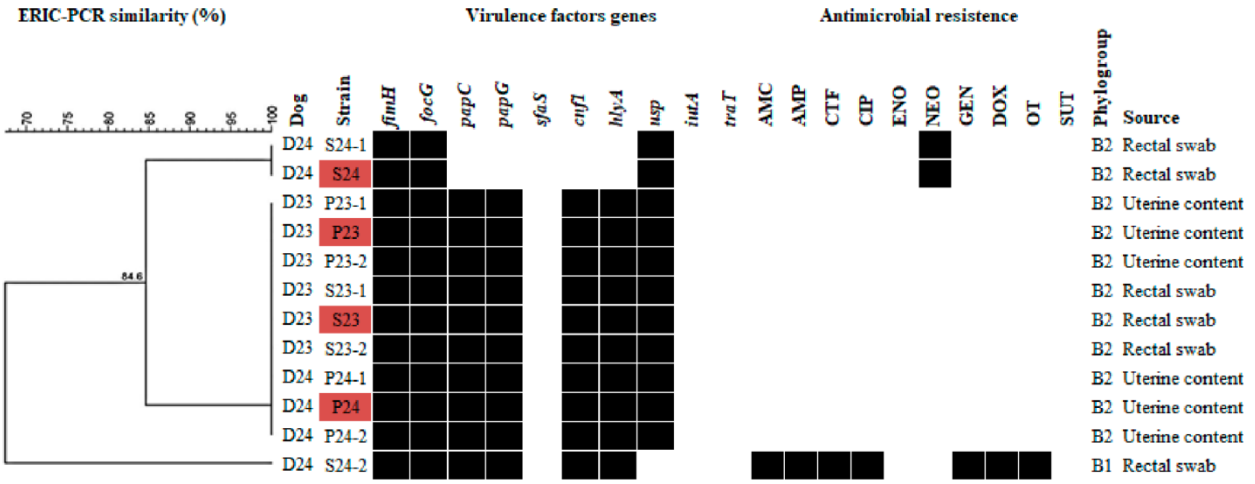

Figure 9. Enterobacterial repetitive intergenic consensus - polymerase chain reaction (ERIC-PCR) similarity, virulence factors, phylogroup, and antimicrobial profile of Escherichia coli isolated from rectal swabs and uterine contents of two cohabiting bitches bitches (D23 and D24). P: uterine content; S: rectal swab, AMC: amoxicillin/clavulanic acid, AMP, ampicillin, CTF: ceftiofur, CIP: ciprofloxacin, ENO: enrofloxacin, NEO: neomycin, GEN: gentamicin, DOX: doxycycline, OT: oxytetracycline, and SUT: trimethoprim/sulfamethoxazole. Isolates marked in red were selected for whole genome sequencing.

Interestingly, the *E. coli* isolates recovered from the D23 rectal swab showed the same phylogroup, virulence factors, and antimicrobial profile as the isolates recovered from the uterus of D23 and D24. In contrast, the strains isolated from the D24 rectal swab differed in phylogroup (one isolate), virulence factors (all three isolates), and antimicrobial susceptibility (all three isolates). Based on these results, two hypotheses were raised: first, that the *E. coli* strain colonizing D23′s rectum ascended to the uterus of this animal, causing the infection; second, that this *E. coli* strain was transmitted to D24, causing pyometra.

To further investigate the possible clonal origin of the isolates, six *E. coli* strains from each dog (tree per isolation) were fingerprinted by enterobacterial repetitive intergenic consensus - polymerase chain reaction (ERIC-PCR) (Lim et al., 2005; Smith et al., 2011) and random repetitive extragenic palindromic (REP)-PCR (Duan et al., 2009; Henriques et al., 2014). These techniques were successfully used in previous investigations of outbreaks caused by

Enterobacteriaceae (Bakhshi et al., 2018). Furthermore, both ERIC-PCR and REP-PCR suggested that all isolates from the uterine content of both dogs and the rectal swab of D23 were 100% similar, but different from all isolates from the rectal swab of D24 (similarity ≤ 84.6%) (Figure 9), reinforcing the suspicion that *E. coli* was transmitted from D23 to D24, causing pyometra.

To better evaluate the hypothesis of transmission of *E. coli* associated with pyometra between the two bitches, four strains were subjected to whole-genome sequencing, currently considered the technique with the highest accuracy and resolution for these cases (Dale and Woodford, 2015). One isolate from each site (uterus and rectum) from each animal was included (marked in red in Figure 9). Genomic deoxyribonucleic acid was extracted using the Maxwell 16® Research Instrument (Promega, Madison, WI, USA) combined with lysozyme (10 mg/mL) and proteinase K (20 mg/mL). The genome was sequenced using the Ion Torrent PGMTM in a mate-pair sequencing kit with an insert size of 3 kbp (~144-fold coverage) and with a fragment sequencing 400 bp kit (~318-fold coverage). The quality of the raw data was analyzed using FastQC (Babraham Bioinformatics), trimming of reads to remove adapters and 3′ ends with Phred's quality score <20 was conducted with an in-house-script (available at https://github.com/aquacen/fast_sample), and assembly was performed using SPAdes 3.5.0 (Nurk et al., 2013). With default parameters, automatic annotation was performed using Prokka 1.10 (Rapid Bacterial Genome Annotation) software (Seemann, 2014). VirulenceFinder 2.0, ResFinder 4.1, and SerotypeFinder 2.0 (Camacho et al., 2009; Joensen et al., 2014; Joensen et al., 2015; Bortolaia et al., 2020; Malberg Tetzschner et al., 2020) were used to identify virulence factors, antimicrobial resistance genes, and mutations, and to predict the O serotype. Multilocus sequence typing (MLST) 2.0 was used to determine sequencing types (ST) according to the Achtman MLST scheme (Wirth et al., 2006; Jaureguy et al., 2008; Camacho et al., 2009; Larsen et al., 2012) and Phyloviz v 2.0, using the goeBURST algorithm (Francisco et al., 2009; Nascimento et al., 2017), was used to infer the population structure with clonal complexes (CCs) composed of all strains sharing at least six identical alleles (single-locus variant). Whole-genome MLST of the four isolates was performed using Ridom SeqSphere+ 4.1.9 (Jünemann et al., 2013) and 13 reference *E. coli* strains from previous studies on humans were included for comparison purposes. These strains were subjected to single-nucleotide polymorphism (SNP) analysis using

CLC Workbench software v 6 (Qiagen, Aarhus, Denmark). Parameters for alignment were settled as mismatch cost = 2; insertion/deletion cost = 1. Parameters to SNP calls were defined to minimum coverage = 10; minimum variant frequency = 50%; filter 454/ion homopolymer indels = 1. Other parameters for alignment and SNP calls remained as the default.

The three likely clonal isolates were classified into a new sequence type on the same CC of ST372 (Figure 10; Table 8), uropathogenic *E. coli* previously described in several reports on dogs and humans, commonly causing genitourinary infection (Wagner et al., 2014; LeCuyer et al., 2018; Flament-Simon et al., 2020; Gilbertie et al., 2020; Kidsley et al., 2020; Valat et al., 2020). Furthermore, the SNP analysis confirmed the clonal relationship between the isolates from the uterine content of D23 and D24 and the strains isolated from the rectum of D23, with a maximum difference of 22 SNPs.

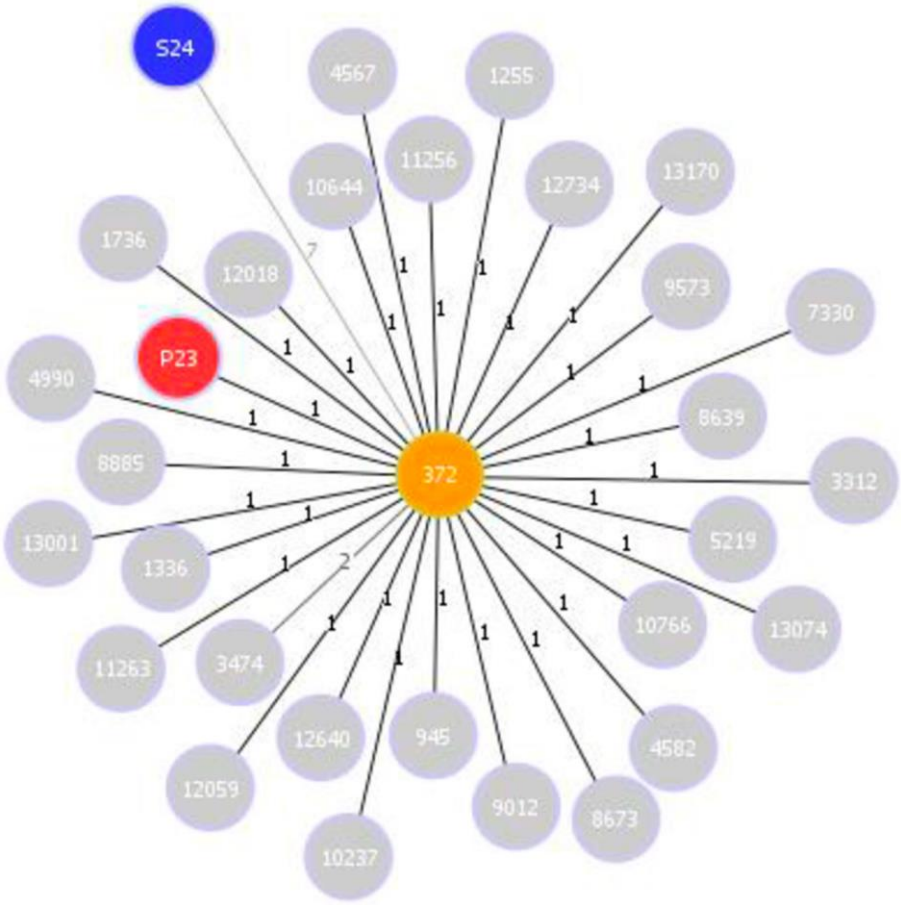

Figure 10. Global optimal - based upon related sequence (goeBURST) population snapshot of clonal complex (CC) 372. Only isolates with a single locus variance (SLV) and isolates from the present study were included: the S24 isolate is marked in blue; ST372 (founder) is marked in orange, P23 is marked in red, and other isolates obtained from the public *E. coli* MLST database are marked in gray; line numbers indicate allelic variance.

Table 8. Results of virulence factors, resistance gene detection, and multilocus sequence typing (MLST) of the four *Escherichia coli* isolates recovered from the uterine contents and rectal swabs of two cohabiting Chow Chow bitches.

Animal	Source	Sample ID	Accession Number	O Serotype[1]	Antimicrobial Resistance Genes	Virulence Factors
D23	Uterine content	P23	JAMJIL000000000	O4:H31	*mdf(A) sitABCD*	*papC, cnf1, focI, hra, papA_F13, focG, usp, chuA, cea, clbB, focCs faE, fyuA, ibeA, iroN, irp2, iss, mchB, mchC, mchF, mcmA, ompT, si*
	Rectal swab	S23	JAMJIK000000000			
D24	Uterine content	P24	JAMJIJ000000000			

						tA, terC, vat, and *yfcV*
	Rectal swab	S24	JAMJII000000000	O5:H22	*mdf(A) sitABCD*	*gad, kpsE, kpsMII, pic, sfaD, tcpC, focG, usp, chuA, cea, clbB, focC sfaE, fyuA, ibeA, iroN, irp2, iss, mchB, mchC, mchF, mcmA, ompT, s itA, terC, vat,* and *yfcV*

[1]Predicted serotype by SerotypeFinder 2.0 (Joensen et al., 2014; Joensen et al., 2015).

Moreover, as expected, the isolate from the rectum of D24 differed greatly from these three isolates (Figure 11). Together, these results confirm that the strain colonizing the rectum of D23 ascended to the uterus of this bitch, causing pyometra. This is the currently accepted pathogenesis of this disease (Hagman, 2022). On the other hand, this same bacterium was later transmitted to the uterus of D24, causing pyometra. This is the first report of a likely transmission of *E. coli* pyometra between two bitches.

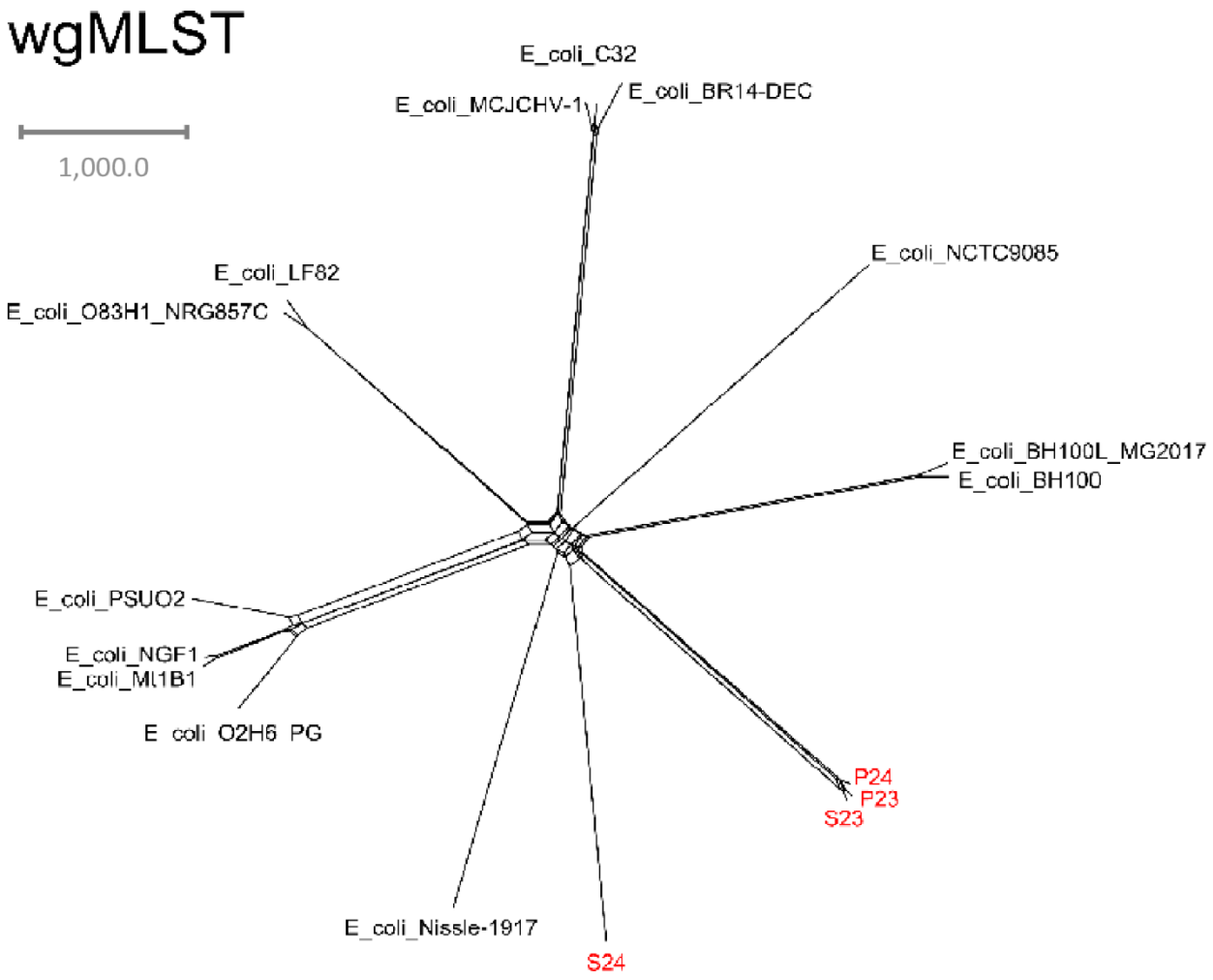

Figure 11. Whole-genome multilocus sequence typing (wgMLST) phylogeny tree including the present study isolates (in red) and reference strains added for comparison purposes.

Despite its clinical relevance, the pathogenesis of canine pyometra remains poorly understood. So far, pyometra has been recognized as a non-transmissible infectious disease (Hagman, 2017; Hagman, 2022). The present study describes a likely transmission of *E. coli* pyometra between two dogs. This hypothesis was first raised after two cohabiting bitches from the same litter developed pyometra in a short period. The genetic similarity seen in the genome of these three strains confirmed the clonal origin, reinforcing the hypothesis of transmission. Although it is impossible to determine the source of contamination for D24, the source was direct or indirect contact with feces or vulvar discharge from D23. Previous studies have suggested that,

in addition to feces, uterine contents may be a source of dissemination of pathogenic *E. coli* to the environment, possibly contaminating other hosts (Coggan et al., 2008; Siqueira et al., 2009; Agostinho et al., 2014). It is worth mentioning that D23 had an open pyometra, and according to the owner, a constant purulent discharge had been observed.

A noteworthy point is that the isolate causing pyometra in both dogs was classified in the same CC of ST372, a well-known pathogen for dogs and humans. In addition, several studies with ExPEC isolated from dogs have shown a high similarity of these isolates with those recovered from humans cohabiting in the same environment (Johnson et al., 2001; Harada et al., 2012; Naziri et al., 2016; Yasugi et al., 2021).

The clone that caused pyometra in both dogs was also isolated from the gut microbiota of dog 23 (D23), but not from the gut of dog 24 (D24). However, it is not possible to completely rule out that this clone was present in the gut of D24. Furthermore, it is also possible that both dogs acquired this clone from an unknown common source. Importantly, these two were the only dogs sharing their environment. Finally, it is also important to consider that some breeds seem to be at increased risk of developing pyometra including Chow Chow (Ewald, 1961; Egenvall et al., 2001; Jitpean et al., 2012).

5.2. Conclusion

For the first time, this study describes the possible transmission of *E. coli* pyometra in dogs. Our findings suggest that in sites with more than one unspayed female, animals with suspected or confirmed pyometra should be isolated from other bitches until clinical resolution. This finding will be relevant to kennel managers, owners with several dogs, and even hospitals.

5.3. Data Availability Statement

The whole-genome shotgun project was deposited in GenBank/NCBI under the BioProject accession number PRJNA824036, Biosample SAMN27382112 (P23), SAMN27382113 (S23), SAMN27382114 (P24), and SAMN27382115 (S24). Genome accession

numbers: JAMJIL000000000 (P23), JAMJIK000000000 (S23), JAMJIJ000000000 (P24), and JAMJII000000000 (S24) [https://www.ncbi.nlm.nih.gov/bioproject/PRJNA824036/, accessed on 8 September 2022].

5.4. Acknowledgments

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6. CHAPTER 4. ASSOCIATION BETWEEN BACTERIAL PATHOGENICITY, ENDOMETRIAL HISTOLOGICAL CHANGES AND CLINICAL PROGNOSIS IN CANINE PYOMETRA

ABSTRACT

Despite the high frequency and clinical relevance of canine pyometra, its pathogenesis remains poorly understood. In this study, the clinical data, histopathological alterations, and microbiological findings of 39 dogs with pyometra were analyzed to assess possible associations. The mean age of the affected animals was 9.6 ± 3.8 years; 76.3% (29/38) had open cervix pyometra, 88% (22/25) had tachypnea, 71% (27/38) had anorexia, and 60.5% (23/38) had leukocytosis. Histopathological analysis revealed that 66.5% (26/39) of the uteri had a high degree of inflammation (score 4). Third-degree hyperplasia of the endometrial epithelium (72%, 28/39) and intralesional or intrauterine bacteria (66.5%, 26/39) were identified in most animals. Bacterial isolates were obtained from 82% (32/39) of the uterine contents and five bacterial species were identified. *Escherichia coli*, classified in phylogroup B2, is associated with virulent adhesion genes (*fimH*, *focG*, and *papC*), and serum resistance (*traT*) was the most common isolate. There was an association between the detection of *papC* in *E. coli* isolates and higher necrosis scores. Additionally, the necrosis score was positively associated with the length of hospitalization, with each point increase in the necrosis score leading to two more days of hospitalization. These results suggest that *papC*-positive *E. coli* play an important role in the severity of pyometra in dogs. The present study revealed the possibility of using this virulence gene to better understand the prognosis of the disease in an affected animal.

Keywords: *Escherichia coli*; canine pyometra; endometrial hyperplasia

6.1. Introduction

Pyometra is the most common reproductive disease in dogs (Hagman, 2023) and is characterized by a suppurative infection with the accumulation of purulent exudates in the uterus (Fieni et al., 2014; Qian et al., 2020). Despite its high prevalence and relevance as a life-threatening disease, the pathogenesis of pyometra remains poorly understood. Studies have suggested factors that predispose patients to the occurrence of the disease, such as age greater than 8 years (Hagman et al., 2011; Jitpean et al., 2012). In addition, some breeds seem more predisposed, such as Boxer, Chow Chow, Cocker Spaniel, Collie, Golden Retriever, Labrador Retriever, Pinscher, Rottweiler, Saint Bernard, and Schnauzer (Rautela and Katiyar, 2019). In addition, dogs with pseudoplacentational endometrial hyperplasia appear to be more predisposed (Santana et al., 2020).

Among the etiological bacteria involved, extraintestinal pathogenic *Escherichia coli* (ExPEC) is by far the most common pathogen isolated in canine pyometra and has been reported in 57%–100% of cases (Chen et al., 2003; Hagman, 2022; Xavier et al., 2022b). These isolates are phylogenetically and epidemiologically distinct from *E. coli* strains found in the intestine as commensals or those that cause gastrointestinal disorders (Tenaillon et al., 2010; Coura et al., 2018; Xavier et al., 2022b). In canine pyometra, *E. coli* strains found in the uterine contents are commonly associated with a specific phylogroup (B2) and have several virulence factors that enhance the colonization of extraintestinal sites, including mostly adhesins and toxins (Henriques et al., 2014; Liu et al., 2017; Xavier et al., 2022b). These virulence factors are believed to play key roles in the development of canine pyometra (Mateus et al., 2013; Henriques et al., 2014; Xavier et al., 2022b). However, it is largely unknown how these ExPEC virulence traits are linked to lesions and clinical severity of pyometra. Therefore, the objective of this study was to verify the associations among clinical data, different degrees of endometrial lesions, and bacterial pathogenicity in bitches with pyometra.

6.2. Materials and methods

6.2.1. Animals

A total of 39 bitches that underwent ovariohysterectomy surgery at the Veterinary Hospital of the Universidade Federal de Minas Gerais (VH-UFMG) were included in this study. Immediately after surgery, the purulent uterine content was sampled, and uterine tissue samples were collected for histopathology. This study was approved by the Ethics Committee on Animal Use of UFMG (Protocol No. 51/2015).

6.2.2. Clinical and epidemiological data

The following data were obtained from the medical clinical records analyzed during the medical consultation, when available: breed, age, type of pyometra, previous use of exogenous progesterone, rectal temperature, respiratory frequency, anorexia, occurrence of vomiting and diarrhea, hemogram, leukogram, renal function, antimicrobial treatment, length of hospitalization, total time of hospitalization, and outcome (Table 9).

6.2.3. Uterine histopathological analyses

Uterine samples were fixed in 10% neutral buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin (HE) (Prabhakaran et al., 2022), followed by microscopic evaluation. Based on a previously described method (Santana et al., 2020; Santana and Santos, 2021), each sample received semi-quantitative scores according to lesion characteristics and intensity for the following lesions: degree of necrosis (score 0 to 3), inflammation (score 0 to 4), hyperplastic changes (endometrial gland ectasia [CEH] (score 0 to 3), pseudoplacentational hyperplasia of the endometrial epithelium [PEH] (score 0 to 3)), and qualitative evaluation for bacterial presence. Details of the parameters used in the present study are provided in Supplementary Table 1.

6.2.4. Bacterial isolation and identification

The uterine contents were streaked on two plates with Mueller-Hinton agar (Kasvi, Italy) supplemented with equine blood (5%) and one plate with MacConkey agar (Difco, USA). The plates were incubated at 37°C for 48 h under aerobic and anaerobic conditions. Isolates identified as *E. coli* were subjected to species-specific polymerase chain reaction (PCR) to confirm its identity (McDaniels et al., 1996). The identities of the other isolates were confirmed by matrix-assisted laser desorption ionization-time of flight (MALDI-ToF) mass spectrometry (Bruker Daltonics, Bremen, Germany), as previously described (Assis et al., 2017; Viegas et al., 2022). Briefly, for each isolate, approximately 1 μL of formic acid (70%) and 1 μL of a saturated solution of α-cyano-4-hydroxycinnamic acid were applied to the spot and allowed to air dry. Spectra were acquired using a FlexControl MicroFlex LT mass spectrometer with a 60-Hz nitrogen laser and approximately 240 laser shots. Parameters for mass range detection were defined as follows: ion source 1 voltage was 19.99 kv, ion source 2 voltage was 18.24 kv, and lens voltage was 6.0 kv for data acquisition, allowing the identification from 1,960 to 20,137 m/z. *E. coli* DH5 alpha was used for calibration and scores ≥ 2.3 were used for a species-level identification as recommended by the manufacturer.

6.2.5. Characterization and virulence genotyping of *E. coli* isolates

E. coli isolates were subjected to previously described PCRs to determinate the phylogroup (A, B1, B2, C, D, E, F, or clade I) (Clermont et al., 2013) and virulence factors corresponding to the ExPEC pathotype: fimbriae type P (*papC* and *papG* allele II and III), fimbriae type I (*fimH*), fimbriae type I central region (*focG*), fimbriae type S (*sfaS*), cytotoxic necrotizing factor type 1 (*cnf1*), uropathogenic specific protein (*usp*), α-hemolysin (*hlyA*), aerobactin (*iutA*), and serum resistance (*traT*) (Johnson and Stell, 2000; Siqueira et al., 2009).

6.2.6. Statistical analysis

The results were analyzed using EngineRoom software (MoreSteam, 2009). To analyze the association between bacterial species, *E. coli* characteristics, histopathological analyses, and clinical data, a multiple-proportion comparison test was performed. This test is based on a chi-

square distribution and a pooled estimate of the population proportion to estimate the standard error of the test statistic. If a significant difference was found in the overall test, a pairwise comparison method with the Marascuilo Procedure was used to identify the specific pairs of proportions that differed significantly. Statistical significance of the results was set at $p \leq 0.05$ for the analyzed characteristics (Marascuilo, 1966).

6.3. Results

6.3.1. Clinical metadata

The availability of clinical information varied between the groups (Table 9). The age of the animals ranged from 3 to 20 years (mean and standard deviation 9.6 ± 3.8 years) and most clinical presentation of pyometra were open cervix (76.3%). Previous use of exogenous progesterone was confirmed in four (11.7%) animals. Most animals had tachypnea (88%), anorexia (71%), or leukocytosis (60.5%). The median length of hospitalization was 2.4 days (range, 0–7 days) and only one death was recorded (2.6%).

Table 9. Clinical and laboratory variables of the female dogs with pyometra.

Variable	Total
Age (years: n=39)	
Mean [Mín.–Máx. ±SD]	9.6 [3–20±3,8]
Previous use of exogenous progesterone (n=34)	
Yes – n (%)	4 (11.7)
Breed (n=39)	
Mixed-breed – n (%)	6 (15.6)
Yorkshire Terrier – n (%)	5 (13)
Poodle – n (%)	4 (10.4)
Golden Retriever – n (%)	3 (7.8)
Labrador Retriever – n (%)	3 (7.8)
Pinscher – n (%)	3 (7.8)
Rottweilers – n (%)	3 (7.8)
Others[1] (%)	12 (31.2)

Pyometra (n=38)	
Open cervix – n (%)	29 (76.3)
Closed cervix – n (%)	9 (23.7)
Hyperthermia (RV[3]: 37.2–39ºC: n=35)	
Yes – n (%)	3 (8.5)
Tachycardia (n=32)	
Yes – n (%)	14 (43.7)
Tachypnea (n=25)	
Yes – n (%)	22 (88)
Anorexia (n=38)	
Yes – n (%)	27 (71)
Vomit (n=38)	
Yes – n (%)	9 (23.6)
Diarrhea (n=38)	
Yes – n (%)	10 (26.3)
Anemia (hematocrit: RV: 37–55%: n=38)	
Yes – n (%)	16 (42.1)
Leukocytosis (cells: RV: 6000–17,000 mm^2: n=38)	
Yes – n (%)	23 (60.5)
Azotemia (creatinine: RV: 0.5–1.5 mg/dL: n=38)	
Yes – n (%)	13 (44.8)
Antimicrobial treatment (antibiotic: n=38)	
Amoxicillin/Clavulanic Acid/Metronidazole – n (%)	20 (52.5)
Amoxicillin/Clavulanic Acid – n (%)	7 (18.5)
Cephalexin – n (%)	4 (10.5)
Cephalexin/ Metronidazole – n (%)	3 (7.5)
Others[2]	4 (10)
Length of hospitalization (days: n=39)	
Mean [Min.–Max.]	2.4 [0–7]
Death (n=34)	
Yes – n (%)	1 (2.6)

[1]Other breeds: American Pit Bull Terrier, Chow Chow, German Shepherd, Lhasa Apso, Border Collie, Maltese, Pekingese, Shih Tzu. [2]Other antimicrobial treatments included cephalexin/clindamycin/enrofloxacin, clindamycin, doxycycline/enrofloxacin, and doxycycline. [3]RV: reference values.

6.3.2. Classification of the endometrial histopathological lesions

Histopathological analysis (Table 10; Figures 12 and 13) revealed that most uteri (26/39, 66.5%) had a high degree of inflammation (score 4). A score 3 of a decidual reaction and hyperplasia of the endometrial epithelium and superficial endometrial glands with moderate to

severe ectasia of the deep endometrial glands and intralesional bacteria were identified in most animals (72% and 66.5%, respectively), and 41% (16/39) of the uteruses had no necrosis (score 0). Endometrial hemorrhage was identified in 46.1% (18/39) of the uteri, with intensities ranging from mild (11.1%, 2/18) to moderate (33.3%, 6/18) to severe (25.6%, 10/18) (Figure 13).

Table 10. Lesion degrees based on the histopathological analyses: inflammation, necrosis, hyperplastic changes, and bacterial presence endometrial in uterus samples of canine pyometra.

Score	Total cases (%)
Inflammation	
0	2 (5)
1	1 (2.5)
2	1 (2.5)
3	9 (23)
4	26 (66.5)
Necrosis	
0	16 (41)
1	10 (25)
2	10 (25)
3	3 (7.5)
Cystic endometrial hyperplasia	
0	36 (92.5)
1	3 (7.5)
2	0
3	0
Pseudo-placentational endometrial hyperplasia	
0	8 (20)
1	3 (7.5)
2	0
3	28 (72)
Intralesional/intrauterine bacteria	
0	13 (33.5)
1	16 (41)
2	10 (25.5)
Total	**39 (100)**

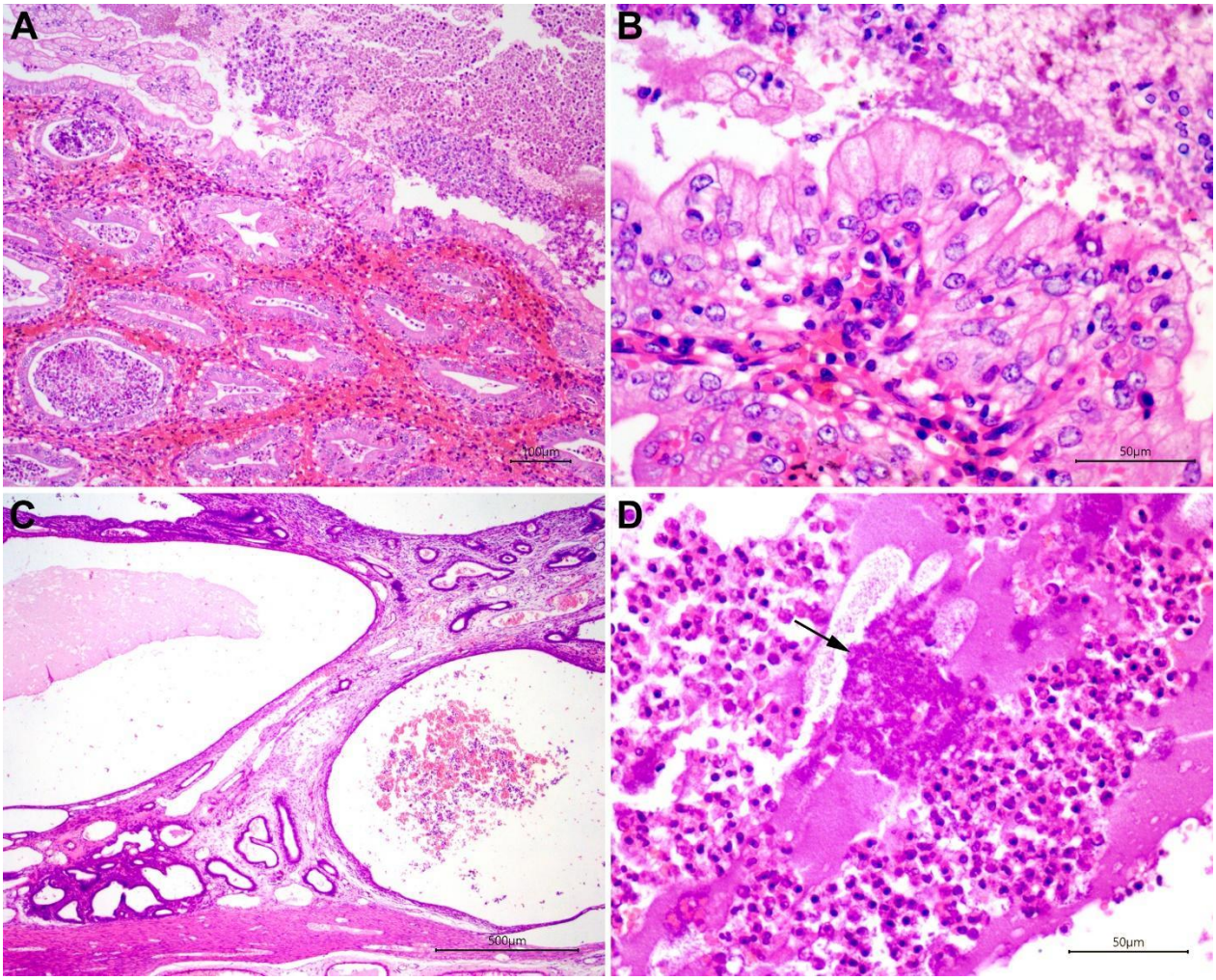

Figure 12. Uterine histopathology of dogs. (A) Uterus with severe inflammation (score 4) and pseudoplacentational endometrial hyperplasia (PEH) (score 3). Inflammation is composed of interstitial and luminal infiltration of large numbers of neutrophils, with cellular debri, endometrial hemorrhage, and ectasia of endometrial glands. HE, scale bar = 100 µm. (B) Uterus with severe inflammation (score 4) and PEH (score 3), with a columnar and finely vacuolized luminal epithelium. HE, scale bar = 50 µm. (C) Uterus with marked inflammation (score 3) and cystic endometrial hyperplasia (CEH) (score 3), characterized by severe glandular ectasia. HE, scale bar = 500 µm. (D) Uterine luminal contents with bacterial aggregates (arrow) and large numbers of neutrophils and some erythrocytes. HE, scale bar = 50 µm.

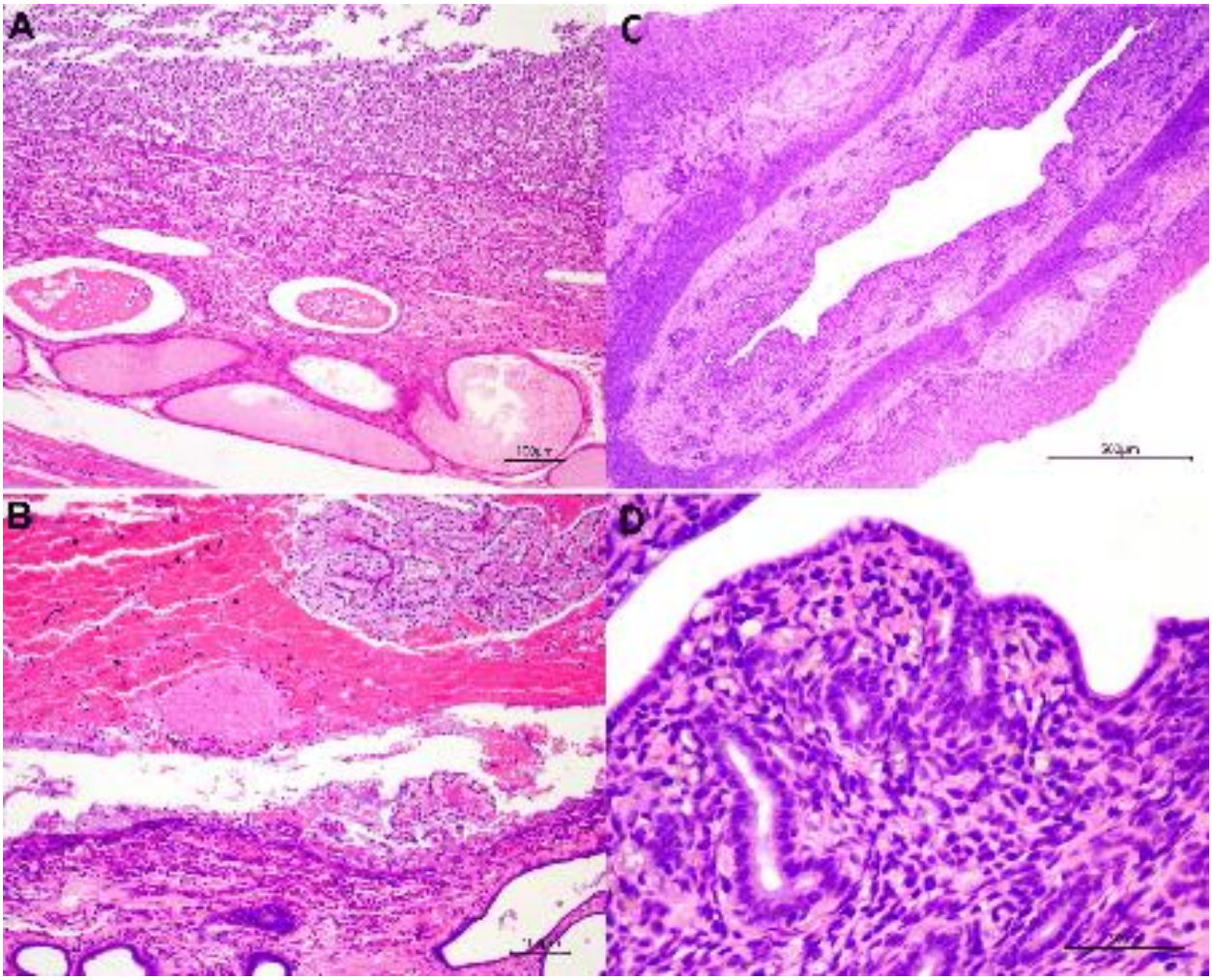

Figure 13. Canine pyometra (A and B) and canine uterus (C and D). A) Uterus with severe inflammation (score 4) and necrosis (score 3), characterized by loss of some superficial endometrial glands and complete loss of endometrial epithelium. HE, scale bar = 100 µm. (B) Uterus with marked inflammation (score 3), PEH (score 3), and severe intraluminal hemorrhage with fibrin deposition. HE, scale bar = 100 µm. (C) Uterus with an empty lumina. Endometrium, myometrium and perimetrium with normal thickness and superficial and deep glands with normal diameter. HE, scale bar = 500 µm. (D) Endometrium with cuboidal luminal epithelium, superficial and deep glands with normal diameter lined by a cuboidal epithelium. Lamina propria with normal connective tissue. HE, scale bar = 50µm.

6.3.3. Bacterial isolates, phylogroup, and virulence factors of *E. coli* isolates

Bacterial isolates were cultured from 82% (32/39) of the intrauterine contents. Of these 32 culture-positive samples, five different bacterial species were identified (Table 11), with *E. coli* 75% (24/32) being significantly the most frequently isolated species (p=0.0001). No mixed bacterial infections were identified.

Table 11. Bacterial species isolated from the intrauterine content of dogs with pyometra.

Organism	Total cases (%)
Escherichia coli	24 (61.5)
Streptococcus canis	3 (7.5)
Enterobacter cloacae	2 (5)
Proteus mirabilis	2 (5)
Klebsiella pneumoniae	1 (2.5)
No growth	7 (17.5)
Total	39 (100)

6.3.4. *E. coli* phylogroup and virulence genes

Three phylogroups were identified among the *E. coli* isolates (Table 12). A total of 83.3% (20/24) of the isolates were classified into phylogroup B2, which was the most frequent (p<0.00001). Phylogroups A, C, D, and E were not identified in this study.

Table 12. Number of isolates and frequency of *Escherichia coli* phylogroups identified in the intrauterine content of dogs with pyometra.

Phylogroup	Total cases (%)
B2	20 (83.2)
B1	1 (4.2)
F	1 (4.2)
Not classified[1]	2 (8.4)
Total	**24 (100)**

[1]Identified as *E. coli* but not corresponding to any of the phylogroups (Clermont et al., 2013).

All tested virulence genes were detected in *E. coli* isolates from intrauterine contents at different frequencies (Table 13). Virulence genes associated with adhesion (*fimH*, 100%; *focG*, 66.5%; *papC*, 50%) and serum resistance (*traT*, 62.5%) were detected in most *E. coli* isolates.

Table 13. Number of isolates and frequency of *Escherichia coli* virulence genes identified in the intrauterine content of dogs with pyometra.

Virulence factors	Total cases (%)
Adhesion	
Fimbriae type I (*fimH*)	24 (100)
Fimbriae type I central region (*focG*)	16 (66.5)
Fimbriae type P (*papC*)	12 (50)
Fimbriae type P (*papG* allele II and III)	8 (33)
Fimbriae type S (*sfaS*)	7 (29)
Toxins	
α-hemolysin (*hlyA*)	11 (46)
Cytotoxic necrotizing factor type 1 (*cnf1*)	9 (37.5)
Uropathogenic specific protein (*usp*)	9 (37.5)
Iron acquisition	
Aerobactin (*iutA*)	11 (46)
Serum resistance	
Serum resistance (*traT*)	15 (62.5)
Total	**24 (100)**

6.3.5. Associations between clinical, histological, and microbial findings

An association between the isolation of *E. coli* positive for the adhesin-encoding gene *papC* and a higher degree of uterine necrosis was observed (p=0.03). In addition, the degree of necrosis was associated with the length of hospitalization (p<0.034): each increase in the degree of uterine necrosis represented an increase in two days of hospitalization, suggesting that higher degrees of necrosis corresponded to more severe clinical manifestations in the dogs, resulting in a longer hospital stay. No other associations between the clinical, histological, and bacterial findings were observed in the present study.

6.4. Discussion

(Pailler et al., 2022)Pyometra is the most frequent reproductive disease in dogs. Despite its known relevance, there are very few studies comparing the clinical, pathological, and microbiological data of affected animals, which can lead to a better understanding of the pathogenesis of this disease. Our results suggest that *E. coli* positivity for a specific fimbria (*papC*) can directly affect uterine necrosis, increasing the hospitalization of the affected animal.

In this study, the mean age of the sampled female dogs (approximately 9 years) was similar to that of previous studies, reinforcing the hypothesis that dogs older than eight years have a higher risk of pyometra (Pailler et al., 2022). Although the effect of age is not fully understood, several hypotheses have been proposed, including immunosenescence, reduction in the capacity of defeat infections, and repeated estrous cycles over the years, which would repeatedly cause elevated estrogen levels, leading to endometrial proliferation (Jitpean et al., 2012; Alexander et al., 2018; Lansubsakul et al., 2022). The use of steroid hormones to prevent pregnancy is also a known predisposing factor (Gibson et al., 2013; Hui et al., 2017; Rungphattanachaikul et al., 2021). In the present work, 1 in 10 owners confirmed the use of this medication, a frequency lower than that reported in other studies (Niskanen and Thrusfield, 1998; Igna et al., 2011). However, it is not possible to state that all veterinarians questioned the owners regarding their previous use of exogenous progesterone during anamnesis, which may have influenced the frequency.

The most common clinical signs of pyometra observed in the present study were purulent vulvar discharge (open cervical pyometra), tachypnea, and anorexia, which were present in more than 71% of the animals, similar to the findings of other studies (Jitpean et al., 2014b; Jitpean et al., 2017; Hagman, 2022). Other clinical signs, including hyperthermia, tachycardia, vomiting, and diarrhea, were found at different rates (between 8.5% and 88%) and reflected the systemic involvement of the disease (Ros et al., 2014; Rungphattanachaikul et al., 2021; Lansubsakul et al., 2022).

Leukocytosis (60.3%), azotemia (44.8%), and anemia (42.1%) were also frequently observed in affected dogs, corroborating previous studies (Fransson et al., 2007; Maddens et al., 2010; Jitpean et al., 2014a; Hagman, 2018). Anemia occurs due to uterine hemorrhage, which is commonly associated with inflammatory exudates in cases of pyometra. Uterine hemorrhage was

diagnosed in 46.1% of the dogs sampled in this study. In addition, suppression of the bone marrow by endotoxins produced by gram-negative bacteria, mainly *E. coli*, can also aggravate anemia. Endotoxemia is also responsible for the intense leukocytosis typically seen in these cases (Martins et al., 2015; Anjos et al., 2021) and can also contribute to kidney damage together with immune complex deposition in the renal glomeruli, leading to azotemia and possible multiple organs dysfunction (Maddens et al., 2010; Anjos et al., 2021; Hagman, 2023).

The length of hospitalization, which is commonly used as a nonspecific indicator of the severity of many diseases (Fransson et al., 2007), was quite low (2.4 days). Previous studies have suggested that up to two days of hospitalization is usually sufficient in uncomplicated cases, suggesting that most dogs included in the present study fall into this classification (Fransson et al., 2007; Hagman, 2022). Corroborating these assumptions, the lethality rate (2.6%) was also low in comparison to previous studies (up to 20%) (Jitpean et al., 2014b; Hagman, 2022). One hypothesis for the low severity of the cases included in this study was the high proportion of open cervical pyometra (76.3%). It is known that the drainage of uterine reduce the risk of complications, including life-threatening conditions like septicemia (Martins et al., 2015; Jitpean et al., 2017).

High scores (scores 3 and 4) for inflammation (89.5%) were observed in the histopathological analyses, and hyperplastic lesions were present in many female dogs (87%), similar to the findings of a recently published study (Lopes et al., 2021). However, since CEH and PEH are two different hyperplastic conditions (Schlafer and Gifford, 2008), these lesions were separated, and PEH (72%) was more frequent than CEH (7.5%) in female dogs with pyometra. These results are consistent with a previous study (Santana et al., 2020) and supports the notion that instead of which was proposed (Dow, 1959), pyometra is more frequently associated to PEH than CEH. Endometrial necrosis observed to varying degrees (57.5%) can be caused by bacterial toxins, in addition to the neutrophilic inflammatory response (Hagman, 2022).

As expected, *E. coli* was isolated from most of the uterine contents of the dogs with pyometra (61.6%). This result is consistent with previous studies showing that *E. coli* is the main bacterium involved in pyometra (Coggan et al., 2008; Hagman, 2018; Anjos et al., 2021; Xavier

et al., 2022b). To better understand the molecular characteristics of *E. coli* involved in pyometra, all isolates recovered in the present study were subjected to phylogroup identification and virulence factor detection, which have been widely used to better understand the colonization dynamics of this bacteria (Clermont et al., 2013; Clermont et al., 2015; Müştak et al., 2015). In the present study, B2 was the most frequent phylogroup, accounting for 83.2% of *E. coli* isolates. This frequency is similar to that reported in previous studies on canine pyometra, suggesting a high capacity of phylogroup B2 strains to colonize the uterus (Mateus et al., 2013; Henriques et al., 2014; Lopes et al., 2021; Xavier et al., 2022b).

Another interesting aspect of *E. coli* is the presence of virulence factors that enable infection of different tissues and sites (Johnson and Stell, 2000; Siqueira et al., 2009). Virulence factors that promote adhesion and colonization, particularly fimbriae, are considered of great relevance in the establishment of *E. coli* infections in the canine uterus (Krekeler et al., 2012; Krekeler et al., 2013; Agostinho et al., 2014; Xavier et al., 2022a). In the present study, three adhesin-encoding virulence genes (*fimH*, *focG*, and *papC*) were the most commonly detected virulence factors, corroborating previous works (Mateus et al., 2013; Henriques et al., 2014; Lopes et al., 2021; Xavier et al., 2022b). Interestingly, the present study revealed an association between *E. coli* positive for *papC* with an increased uterine necrosis. Additionally, the degree of necrosis was positively associated with the length of hospital stay, with each increase in the degree of necrosis representing approximately two more days of hospitalization. Taken together, these findings reinforce the hypothesis that *papC* is strongly associated with pyometra caused by *E. coli*. In fact, a previous study showed that this virulence factor is directly associated with the adhesion and colonization of the canine endometrium and seems to facilitate the migration of bacteria present in the intestinal tract to the canine uterus, causing infection (Krekeler et al., 2013). Our findings also support the idea that higher tissue necrosis, associated with the presence of *papC*, can directly impact disease severity, increasing the hospitalization time required. This is the first study to report an association between bacterial virulence factors and histopathological changes, as well as clinical outcomes.

6.6. Supplementary data

Supplementary table 1. Characteristics and intensities of each score classification based on the observed lesions including necrosis, inflammation, hyperplastic changes (cystic endometrial hyperplasia [CEH] and pseudoplacentacional endometrial hyperplasia [PEH]) and bacterial presence at uterine histopathological analyses.

Lesions classifications	Score Description
Necrosis	
0	Absence of necrosis.
1	Multifocal areas (up to 30%) of necrosis of endometrial epithelium from uterine fragments.
2	Multifocal areas (30 to 70%) of endometrial epithelium necrosis of uterine fragments.
3	Necrosis and loss of the entire endometrial epithelium, in at least half of the uterine fragments evaluated.
Inflammation	
0	Absence of inflammation.
1	Inflammatory lympho-histioplasmacytic infiltrate, with or without neutrophils, in the endometrium interstitium.
2	Lympho-histioplasmacytic inflammatory infiltrate, with or without neutrophils in the endometrium interstitium, with neutrophil exocytosis in the glandular and/or endometrial epithelium, and/or with rare (1 to 3) glands with intraluminal neutrophils.
3	Lympho-histioplasmacytic inflammatory infiltrate, with or without neutrophils, in the endometrial interstitium, with intense neutrophilic infiltrate in the uterine lumen and/or glandular.
4	Lympho-histioplasmacytic inflammatory infiltrate, with or without neutrophils, extending from the endometrium to the myometrium, with intense neutrophilic infiltrate in the uterine lumen and/or glandular.
Hyperplastic changes (CEH)	
0	No CEH hyperplastic process.
1	Mild endometrial gland ectasia, with endometrial and glandular cuboidal epithelium with homogeneous cytoplasm.
2	Moderate endometrial gland ectasia, with endometrial and glandular cuboidal epithelium with homogeneous cytoplasm.
3	Marked endometrial gland ectasia, with endometrial and glandular cuboidal epithelium with homogeneous cytoplasm.
Hyperplastic changes (PEH)	
0	Absence of PEH hyperplastic process.
1	Decidual reaction and mild hyperplasia of the endometrial epithelium, with or without ectasia of endometrial glands.
2	Decidual reaction and moderate endometrial epithelial hyperplasia, with or without endometrial gland ectasia.
3	Decidual reaction and hyperplasia of the endometrial and superficial endometrial glands epithelium with moderate to severe ectasia of deep endometrial glands.
Presence of bacteria	
0	Absence of bacteria in the uterine contents and in endometrial glands lumina.
1	Myriad of bacteria in the contents of the uterine lumen.
2	Bacterial myriad in the contents of the uterine lumen and in the endometrial glands lumina.

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6.8. References

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7. CONCLUSIONS

The present work suggests that intestinal colonization by *E. coli* from phylogroup B2 may be a risk factor for the occurrence of *E. coli* pyometra in bitches. Also, the present study indicates that the frequency of *E. coli* from phylogroup B2 can vary according to the diet. Further studies are necessary to verify how diet modulates intestinal colonization by *E. coli*, which can be a future tool to reduce the occurrence of *E. coli* pyometra in dogs.

For the first time, this study describes the possible transmission of *E. coli* pyometra in dogs. Our findings suggest that, in locations with more than one non-spayed bitches, animals with suspected or confirmed pyometra should be isolated from other animals until clinical resolution. This discovery will is of importance for kennels, multiple dog owners, hospitals, among other places where more than one female dog cohabiting.

Finally, the association between a specific *E. coli* virulence factor, uterine necrosis and length of hospital stay reinforce the hypothesis that some bacterial strains directly impact the severity of the disease. This finding improved the knowledge regarding the pyometra pathogenesis.

8. ATTACHMENTS

8.1. Published articles and thesis products

8.1.1. Articles in scientific journals

XAVIER, R.G.C.; SILVA, P.H.S. DA; TRINDADE, H.D.; CARVALHO, G.M.; NICOLINO, R.R.; FREITAS, P.M.C.; SILVA, R.O.S. Characterization of Escherichia coli in Dogs with Pyometra and the Influence of Diet on the Intestinal Colonization of Extraintestinal Pathogenic E. coli (ExPEC). **Veterinary Sciences**, v.9, p.245, 2022b. DOI: 10.3390/vetsci9050245.

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XAVIER, R.G.C.; SANTANA, C.H.; SILVA, P.H.S. DA; PARAGUASSÚ, A.O.; NICOLINO, R.R.; FREITAS, P.M.C.; SANTOS, R. DE L.; SILVA, R.O.S. Association between bacterial pathogenicity, endometrial histological changes and clinical prognosis in canine pyometra. **Theriogenology**, v.214, p.118–123, 2023b. DOI: 10.1016/j.theriogenology.2023.10.007.

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8.1.4. Awards and titles

Honorable Mention for the presentation of the work entitled Primeiro Relato de Transmissão de Piometra entre Duas Cadelas Coabitantes, UniBH - Ecossistema Ânima. 2022.

Academic relevance for the presentation of the work entitled Primeiro Relato de Transmissão de Piometra entre Duas Cadelas Coabitantes, UniBH - Ecossistema Ânima. 2022.

veterinary sciences

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Article

Characterization of *Escherichia coli* in Dogs with Pyometra and the Influence of Diet on the Intestinal Colonization of Extraintestinal Pathogenic *E. coli* (ExPEC)

Rafael Gariglio Clark Xavier, Paloma Helena Sanches da Silva, Hanna Dornelas Trindade, Gabriela Muniz Carvalho, Rafael Romero Nicolino, Patrícia Maria Coletto Freitas and Rodrigo Otávio Silveira Silva *

Affiliation Veterinary School, Federal University of Minas Gerais, Antônio Carlos Avenue 6627, 31270-090 Belo Horizonte, Brazil; rafaelgariglio90@gmail.com (R.G.C.X.); palomasanches.vet@gmail.com (P.H.S.d.S.); nanadt@gmail.com (H.D.T.); munizcgabriela@gmail.com (G.M.C.); rafael.nicolino@gmail.com (R.R.N.); pcoletto@yahoo.com.br (P.M.C.F.)
* Correspondence: rodrigo.otaviosilva@gmail.com

Abstract: Despite its high frequency and clinical relevance, the pathogenesis of canine pyometra remains poorly understood. The most accepted hypothesis is that bacteria involved ascend from the intestinal tract, causing the uterine infection. Extraintestinal pathogenic *Escherichia coli* (ExPEC) is the most frequent pathogen in canine pyometra, accounting for 57–100% of cases. The aim of the present study was to determine the frequency of phylogenetic groups and virulence factors in *E. coli* strains isolated from the uterine and rectal swabs of bitches with pyometra (n = 72) and from rectal swabs from healthy bitches fed commercial dry feed (n = 53) or a raw meat-based diet (RMBD; n = 38). A total of 512 strains of *E. coli* were isolated and divided into five categories according to the origin of the sample: 120 isolates from the uterine content of dogs with *E. coli* pyometra, 102 from the feces of bitches with *E. coli* pyometra, 75 from the feces of bitches without *E. coli* pyometra, 130 feces samples from healthy dogs fed commercial feed, and 85 feces samples from healthy dogs fed a raw meat-based diet. *E. coli* strains belonging to the B2 phylogroup and positive for virulence factor genes associated with adhesion (fimbriae type P [*papC*]) and production of toxins (α-hemolysin [*hlyA*] and uropathogenic specific protein [*usp*]) predominated in the uterine content and rectal swabs of bitches with *E. coli* pyometra. Interestingly, a lower growth rate of *E. coli* from the B2 phylogroup was observed in dogs fed a RMBD than in those fed commercial dry feed. The present study suggests that intestinal colonization by certain types of *E. coli* could be a risk factor for the occurrence of *E. coli* pyometra in bitches and that diet can influence intestinal colonization by such strains.

Keywords: EnPEC; UPEC; RMDB; uterus; uterine; microbiota

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1. Introduction

Pyometra is the most frequently occurring reproductive disease in bitches, affecting up to 25% of uncastrated females [1,2]. The disease is characterized by bacterial infection of the uterus with local and systemic clinical manifestations that can lead to death [3–5]. However, despite its relevance, the pathogenesis of this disease remains poorly understood. It is believed that bacterial species causing pyometra ascend from the intestinal tract of females, causing infections [1,2,5,6].

Extraintestinal pathogenic *Escherichia coli* (ExPEC) is the most common pathogen involved in canine pyometra and has been reported in 57–100% of cases [1,2,6,7]. These isolates are phylogenetically and epidemiologically distinct from *E. coli* strains commonly found in intestinal commensals that cause diarrhea and other gastrointestinal disorders [8–10]. In canine pyometra, *E. coli* strains found in uterine contents are commonly associated with phylogroup B2 and less frequently with phylogroup D [11–13]. In contrast, commensal

intestinal strains of *E. coli* in dogs are mostly classified into phylogenetic groups B1 and A [5,9,12]. In addition, *E. coli* recovered from pyometra have specific virulence factors, such as adhesins, toxins, iron acquisition systems, and protectins [7,14,15], which are commonly classified as endometrial pathogenic *E. coli* (EnPEC), a subgroup of the ExPEC pathotype. These virulence factors may confer a selective advantage over commensal strains [16], playing a key role in the development of canine pyometra [11,14,17] as well as in other extraintestinal infections in humans and animals [16–18].

Although the intestinal ascension of *E. coli* strains is currently the most accepted hypothesis in the pathogenesis of canine pyometra [1,2,7], no studies have evaluated the influence of the dog diet on the specific colonization of ExPEC strains. In the last decade, an increasing number of owners have been feeding their dogs and cats raw meat-based diets (RMBDs), instead of regular commercial dry feed [19]. Dogs fed an RMBD shed an increased amount of some pathogens in their feces, including *Salmonella* spp. and diarrheagenic *E. coli* [19–21]. However, specific virulence factors related to extraintestinal infections have not yet been investigated. The aim of this study was to determine the prevalence of phylogroups and virulence factors in *E. coli* isolates obtained from the uterine contents and feces of bitches with pyometra infection. In addition, we compared these *E. coli* isolates with those obtained from the feces of healthy dogs fed commercial dry feed or an RMBD to evaluate the possible influence of diet on colonization by *E. coli* strains.

2. Materials and Methods

2.1. Sampling

Three groups of bitches were sampled in the present study: dogs with pyometra (uterine and rectal swabs), healthy dogs fed commercial dry feed (rectal swab), and healthy dogs fed a RMBD (rectal swab). A total of 72 bitches with pyometra who underwent ovariohysterectomy (OH) surgery at the Veterinary Hospital of the Universidade Federal de Minas Gerais (VH-UFMG) between January 2017 and December 2020 were included. Immediately following surgery, aspiration puncture of the uterine contents was performed and a swab was introduced into the rectal ampulla of the bitches. The samples were refrigerated at 4 °C until processing for a maximum of 24 h. Rectal swabs from 91 healthy dogs were included, of which 53 were fed commercial dry feed, and 38 were fed a RMBD. The samples were kept in a cooler with ice packs and transported for processing within a maximum of 24 h. This study was approved by the Ethical Committee on Animal Use of UFMG (protocol No. 51/2015).

2.2. Isolation and Identification of E. coli

The uterine contents were plated on Mueller Hinton (MH) agar (Kasvi, Maharashtra, India) supplemented with equine blood (5%) and MacConkey (MC) agar (Difco, Franklin Lakes, NJ, USA), and the plates were incubated at 37 °C for 48 h under aerobiosis and anaerobiosis. Plating of rectal swab samples from female dogs subjected to OH and healthy dogs was performed on MC agar and incubated at 37 °C for 48 h under aerobiosis. For each clinical specimen, up to three lactose-fermenting colonies were subjected to species-specific polymerase chain reaction (PCR) to identify *E. coli* [22]. Strains not identified as *E. coli* was identified by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-ToF MS; Bruker Daltonics, Billerica, USA). A cutoff log score of 2 was used to validate the identification at the species level, as recommended by the manufacturer.

2.3. Characterization of E. coli

E. coli strains were subjected to PCR to determine phylogroups (A, B1, B2, C, D, E, F, or clade I) [23], and identify virulence genes corresponding to the ExPEC pathotype, namely, fimbriae type I (*fimH*), fimbriae type I central region (*focG*), fimbriae type P (*papC* and *papG*, allele II and III), fimbriae type S (*sfaS*), cytotoxic necrotizing factor type 1 (*cnf1*), uropathogenic specific protein (*usp*), α-hemolysin (*hlyA*), aerobactin (*iutA*), and serum resistance (*traT*) [18,24].

2.4. Statistical Analysis

The results were analyzed using EngineRoom software [25]. To analyze the association between *E. coli* phylogroups, virulence factors, and categorical variables related to the group origin of the samples (uterine content of bitches with *E. coli* pyometra, rectal swabs of bitches with *E. coli* pyometra, or without *E. coli* pyometra, and healthy dogs fed commercial dry feed or a RMBD) a multiple proportion comparison test was conducted. This test is based on the chi-square distribution and the pooled estimate of the population proportion to estimate the standard error of the test statistic. If a significant difference was found in the overall test, the pairwise comparisons method with Marascuillo procedure was used to identify the specific pairs of proportions which differ significantly. Statistical significance of the results was set at $p \leq 0.05$ for the analyzed characteristics [26].

3. Results

3.1. E. coli Isolation

A total of 40 (56%) of the 72 dogs tested positive for *E. coli* in the uterine content; up to three colonies were obtained from each, totaling 120 *E. coli* strains, while 21 (29%) had only other pathogens, and no bacterial growth was seen in 11 (15%) (Table 1).

Table 1. Bacterial species isolated from the uterus in bitches with pyometra.

Organism	Total Cases (%)
Escherichia coli	40 (56)
Staphylococcus sp.	6 (8)
Streptococcus sp.	6 (8)
Enterobacter sp.	2 (3)
Enterococcus sp.	2 (3)
Klebsiella pneumoniae	2 (3)
Proteus mirabilis	2 (3)
Pseudomonas aeruginosa	1 (1)
No growth	11 (15)
Total	72 (100)

Up to three colonies of *E. coli* were obtained from rectal swabs of 59 bitches with pyometra, totaling 177 *E. coli* strains: 102 from dogs that tested positive for *E. coli* content (*E. coli* pyometra) and 75 from bitches that tested negative for *E. coli* (without *E. coli* pyometra) in the uterine contents.

From healthy bitches, at least one *E. coli* isolate was recovered from 91 dogs sampled, totaling 215 strains: 130 and 85 from dogs fed commercial feed or RMBD, respectively.

3.2. E. coli Phylogroups

Phylogroup B2 was the most common *E. coli* phylogroup detected in the uterine contents of bitches infected with *E. coli* pyometra (85%) and also in the rectal swab isolates of bitches with *E. coli* pyometra (58.8%), whereas B1 was most frequent in the rectal swabs of bitches without *E. coli* pyometra (41.3%). Bitches with *E. coli* pyometra showed a higher frequency of phylogroup B2 in the rectal swab than females without *E. coli* pyometra ($p < 0.05$). Phylogroup B2 was also the most frequent in *E. coli* isolates from rectal swabs of dogs fed commercial dry feed (34.6%), whereas B1 was the most common in dogs fed RMBD (34.1%). Dogs fed commercial dry feed showed a higher frequency of phylogroup B2 in rectal swabs than dogs fed RMBD ($p < 0.05$) (Table 2).

Table 2. Number of isolates and frequency of *E. coli* phylogroups identified in the uterine content, rectal swabs of bitches with pyometra and rectal swabs of healthy dogs.

	Bitches with Pyometra		Healthy Dogs		
	Uterine Content		Rectal Swab		
Phylogroup	*E. coli* Pyometra	*E. coli* Pyometra	Non-*E. coli* Pyometra	Consume Commercial Dry Feed	Consume RMBD
A	0	2 (1.9%)	5 (6.6%)	4 (3%)	6 (7%)
B1	3 (2.5%)	22 (21.5%)	31 (41.3%)	35 (26.9%)	29 (34.1%)
B2	102 (85%)	60 (58.8%) [a]	18 (24%)	45 (34.6%) [b]	8 (9.4%)
C	0	4 (3.9%)	0	16 (12.3%)	11 (12.9%)
D	0	0	2 (2.6%)	1 (0.7%)	0
E	6 (5%)	7 (6.8%)	6 (8%)	10 (7.6%)	20 (23.5%)
F	3 (2.5%)	3 (2.9%)	10 (13.3%)	11 (8.4%)	10 (11.7%)
E. clades—clade I	0	0	0	3 (2.3%)	0
Not classified	6 (5%)	4 (3.9%)	3 (4%)	5 (3.8%)	1 (1.1%)
Total	120	102	75	130	85

[a] Samples with statistical difference when comparing strains obtained from the rectal swab of bitches with *E. coli* pyometra and bitches with non-*E. coli* pyometra. [b] Samples with statistical difference when comparing strains obtained from the rectal swabs of dogs fed commercial dry food and dogs fed RMBD.

3.3. Frequency of Virulence Genes Associated with the ExPEC Pathotype

All the virulence genes tested were detected in *E. coli* isolates from all groups at different frequencies. Virulence genes associated with adhesion (papC) and toxin production (hlyA and usp) were more frequent in the rectal swabs of bitches with *E. coli* pyometra than in those without *E. coli* pyometra ($p < 0.05$). In addition, two virulence genes associated with adhesion (focG and sfaS) were more frequent in isolates from dogs fed commercial dry feed than in those from dogs fed RMBD ($p < 0.05$). In contrast, the serum resistance gene (traT) was found at a higher frequency in isolates from dogs fed RMBD than in those from dogs fed commercial dry feed ($p < 0.05$) (Table 3).

Table 3. Number of isolates and frequency of *E. coli* virulence genes identified in the uterine content, rectal swabs of bitches with pyometra and rectal swabs of healthy dogs.

	Bitches with Pyometra		Healthy Dogs		
	Uterine Content		Rectal Swab		
Virulence Genes	*E. coli* Pyometra	*E. coli* Pyometra	Non-*E. coli* Pyometra	Consume Commercial Dry Feed	Consume RMBD
Adhesion					
fimH	120 (100%)	102 (100%)	75 (100%)	128 (98.4%)	83 (97.6%)
focG	66 (55%)	56 (54.9%)	25 (33.3%)	47 (36.2%) [b]	15 (17.6%)
papC	66 (55%)	45 (44.1%) [a]	16 (21.3%)	38 (29.2%)	14 (16.4%)
papG	58 (48.3%)	36 (35.2%)	13 (17.3%)	74 (56.9%)	37 (43.5%)
sfaS	27 (22.5%)	16 (15.6%)	4 (5.3%)	26 (20%) [b]	1 (1.1%)
Toxins					
cnf1	50 (41.6%)	33 (32.3%)	14 (18.6%)	20 (15.3%)	9 (10.5%)
hlyA	61 (50.8%)	40 (39.2%) [a]	13 (17.3%)	22 (16.9%)	10 (11.7%)
usp	48 (40%)	28 (27.4%) [a]	4 (5.3%)	16 (12.3%)	5 (5.8%)
Iron acquisition					
iutA	43 (35.8%)	39 (38.2%)	26 (34.6%)	103 (79.2%)	74 (87%)
Serum resistance					
traT	76 (63.3%)	57 (55.8%)	48 (64%)	91 (70%)	84 (98.8%) [b]
Total	120	102	75	130	85

[a] Samples with statistical difference when comparing strains obtained from the rectal swab of bitches with *E. coli* pyometra and bitches with non-*E. coli* pyometra. [b] Samples with statistical difference when comparing strains obtained from the rectal swabs of dogs that consume commercial dry food and dogs that consume RMBD.

4. Discussion

As expected, *E. coli* was isolated from most of the uterine contents of dogs with pyometra. This result is in accordance with previous studies showing that *E. coli* is the main bacterium involved in pyometra [6,27,28].

Differentiation into phylogenetic groups and the detection of virulence factors have been widely used in studies on *E. coli*, helping to elucidate the epidemiology of infections and the colonization dynamics of these bacteria [5,23,29]. Previous studies have demonstrated that ExPEC strains isolated from canine pyometra tend to cluster mainly in phylogroup B2, whereas those isolated from the intestinal microbiota of healthy dogs cluster mainly in phylogenetic groups B1 and A [9,12,30]. In the present study, phylogroup B2 was the most frequent in the uterine contents of bitches, with clinical cases of pyometra caused by *E. coli* (Figure 1), corresponding to 85% of the isolates. This frequency is similar to that found in previous studies on pyometra, suggesting a high capacity of phylogroup B2 strains to colonize the canine uterus [7,11,31].

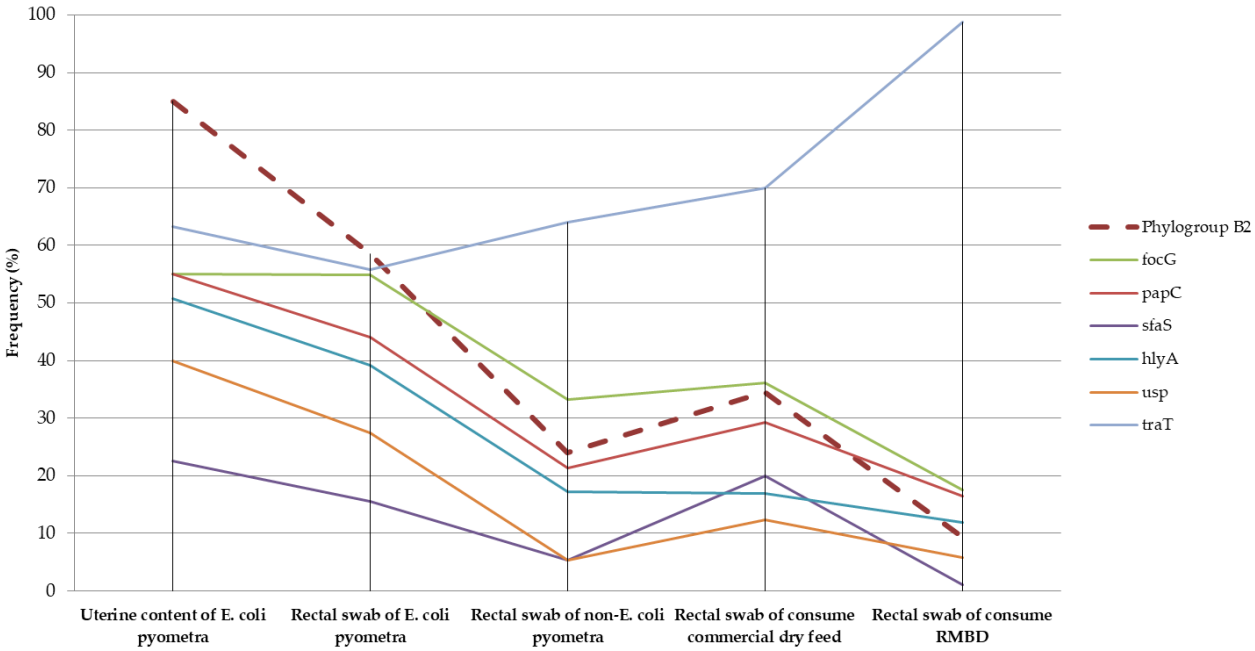

Figure 1. Frequency of the phylogroup B2 and the main virulence factors identified in *E. coli* isolated from the uterine content, rectal swabs of bitches with pyometra and rectal swabs of healthy dogs fed commercial dry feed and raw meat-based diet (RMBD).

Although the pathogenesis of pyometra is poorly understood, previous studies have suggested that the intestine is the main source of *E. coli* strains that ascend into the uterus [1,2,7]. This study reinforces this hypothesis, as bitches with *E. coli* pyometra were more likely to harbor *E. coli* strains from phylogroup B2 in the rectal swab when compared to the group of bitches without *E. coli* pyometra. This finding indicates that intestinal colonization by *E. coli* from phylogroup B2 increases the risk of pyometra in female dogs.

Another interesting aspect of ExPEC is the presence of certain virulence factors that enable infection at different locations [5]. Virulence factors that promote adhesion and colonization, especially fimbriae, are considered to be of great relevance for the establishment of *E. coli* infections in the canine uterus [18,32,33]. Previous studies demonstrated that simple inactivation of some adhesins, such as type 1 (*fim*), P (*papGIII*), and S (*sfa/foc*) fimbriae, results in a considerable reduction in bacterial binding to cell lines of the canine endometrium, reinforcing the importance of these factors in the pathogenesis of the disease [34]. In the present study, four adhesin-encoding virulence genes were found more frequently in *E. coli* samples obtained from the uterine contents, similar to the findings

of previous studies [11,18,31]. This finding reinforces the hypothesis that some adherent virulence factors are associated with pyometra caused by *E. coli* in female dogs. It is noteworthy that the gene encoding type P fimbriae (*papC*), which is considered important for the adhesion and colonization of *E. coli* in the canine endometrium [6,7], was found in 55% of the isolates from the uterine contents. This frequency is similar to that identified in other studies on canine pyometra isolates [5,18]. In addition, strains isolated from the rectal swabs of bitches with *E. coli* pyometra were more commonly positive for the type P fimbriae gene (*papC*) than strains isolated from the rectal swabs of dogs without *E. coli* pyometra. Notably, the frequency of *papC*-positive *E. coli* strains in dogs without *E. coli* pyometra was similar to that reported in a previous study on *E. coli* from rectal swabs of healthy dogs [17].

Although *E. coli* is known to be the main bacterium involved in pyometra [1,2], and recent studies have suggested that diet can influence *E. coli* colonization [8,35,36], current studies have evaluated how different diets would affect the frequency of ExPEC in bitches. In the present study, the *papC* gene showed no statistical difference between the groups of healthy bitches under different types of feeding. In contrast, the genes encoding type 1 adhesin (*focG*) and S (*sfaS*) fimbriae were found less frequently in *E. coli* strains recovered from dogs fed RMBD. These adhesins are considered important in the pathogenesis of canine pyometra [11,32,37]. However, it is important to note that the frequency of these two adhesin-encoding genes was similar in strains isolated from rectal swabs of dogs with or without *E. coli* pyometra, raising doubts regarding the role of these virulence factors in disease development.

Previous studies have indicated that ExPEC obtained from the uterine content of bitches with pyometra commonly expresses genes encoding toxins that may provide a selective advantage [1,2,7,38]. We observed that all toxin-coding virulence genes were found more frequently in *E. coli* samples obtained from the uterine content, which is in agreement with previous studies [6,18,31], which reinforces the hypothesis that, in addition to adhesins, ExPEC toxin virulence factors are associated with the occurrence of *E. coli* pyometra. Among the *E. coli* isolates from rectal swabs, the α-hemolysin (*hlyA*) toxin, which is capable of lysing erythrocytes and leukocytes [31,38,39], was found more frequently in strains isolated from bitches with *E. coli* pyometra than in strains isolated from rectal swabs of dogs without *E. coli* pyometra. Additionally, the uropathogenic specific protein (*usp*), which acts as a bacteriocin and assists in the migration of strains into the bloodstream [18,33,40], was more frequent in strains isolated from the rectal swabs of bitches with *E. coli* pyometra than in strains isolated from dogs without *E. coli* pyometra.

ExPEC obtained from the uterine contents of bitches with pyometra is commonly positive for the aerobactin gene (*iutA*), a virulence factor responsible for iron acquisition [5,38], and for the serum resistance gene (*traT*), a virulence factor associated with the inhibition of the immune response of the host in cases of translocation of the pathogen into the bloodstream [31,39]. In the present study, both virulence genes were detected in all groups, and the frequency was similar among *E. coli* strains obtained from uterine content and rectal swabs from bitches with *E. coli* and without *E. coli* pyometra. In contrast, *traT* was more frequently detected in *E. coli* strains from rectal swabs of dogs fed RMBD than in those fed commercial dry feed.

Research on phylogroups and virulence factors of *E. coli* from different origins has increased over the last few years, but many gaps remain, mostly regarding *E. coli* colonization and infection in dogs [1,7]. In the present study, we demonstrated that, compared to dogs without *E. coli* pyometra, dogs with *E. coli* pyometra are more likely to be colonized by *E. coli* from phylogroup B2, which is positive for specific virulence genes, including type 1 adhesin (*papC*) and two toxins (*hlyA* and *usp*). These results suggest that colonization by these strains is a risk factor for canine pyometra caused by *E. coli*. Based on these results, we sampled two groups of healthy dogs under different diets to evaluate whether dietary habits altered the intestinal microbiota and further established *E. coli* in the B2 phylogroup. Our results suggest that dogs fed RMBD are less frequently colonized by *E. coli* strains from

phylogroup B2, raising the hypothesis that diet can be a risk factor for the occurrence of *E. coli* pyometra, which is the main bacterium responsible for this disease [6,7,41].

Importantly, several studies have indicated public health risks associated with RMBD, such as greater fecal shedding of pathogenic and zoonotic microorganisms, which is a potential risk to animal and human health [19,42]. Therefore, several health agencies have released statements that discourage the inclusion of raw or undercooked animal protein in dog diets [42]. We believe that the results of this study will motivate future evaluations of different diets for dogs that aim to reduce the colonization of ExPEC, however, this study should not be considered as a motivation for the adoption of RMDB, owing to the known risks of this practice.

5. Conclusions

The present study demonstrated the high frequency of *E. coli* strains belonging to phylogroup B2 and carrying virulence factors associated with ExPEC in isolates from the uterine contents of bitches with pyometra. In addition, this study found a higher frequency of these strains in the intestinal microbiota of bitches with *E. coli* pyometra than in bitches without *E. coli* pyometra, suggesting that intestinal colonization by these strains could be a risk factor for the occurrence of *E. coli* pyometra in dogs. Interestingly, when evaluating the intestinal microbiota of dogs on different types of diets, the present study found a lower frequency of such strains in the intestinal microbiota of dogs subjected to a RMBD than in dogs who consumed commercial dry feed, suggesting that future studies on diet modulation affecting intestinal colonization could find mechanisms to prevent and control *E. coli* pyometra in dogs.

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Case Report

Transmission of *Escherichia coli* Causing Pyometra between Two Female Dogs

Rafael Gariglio Clark Xavier, Clarissa Helena Santana, Paloma Helena Sanches da Silva, Flávia Figueira Aburjaile, Felipe Luiz Pereira, Henrique César Pereira Figueiredo, Patrícia Maria Coletto Freitas, Renato Lima Santos and Rodrigo Otávio Silveira Silva *

Veterinary School, Federal University of Minas Gerais, Antônio Carlos Avenue 6627, Belo Horizonte 31270-090, Brazil

* Correspondence: rodrigo.otaviosilva@gmail.com

Abstract: Despite its clinical relevance, the pathogenesis of canine pyometra remains poorly understood. To date, it is recognized as a non-transmissible infectious disease. In this study, the simultaneous occurrence of pyometra and *Escherichia coli* in two cohabitant female dogs underwent in-depth investigation due to the hypothesis of transmission between these animals. Two 5-year-old Chow Chow dogs (namely, dogs 23 and 24—D23 and D24) were referred to a veterinary hospital with suspected pyometra. Both animals showed prostration, anorexia, and purulent vulvar discharge over a 1-week period. After ovariohysterectomy, uterine tissue, uterine contents, and rectal swabs were collected for histopathological and microbiological analysis. Uterine histology demonstrated purulent material and multifocal necrosis with endometrial ulceration, and a morphological diagnosis of pyometra was confirmed. Furthermore, *E. coli* from the same phylogroup (B2) and positive for the same virulence factors with the same antimicrobial susceptibility profile was isolated from the uterine contents of both dogs and the rectum of D23. Conversely, the *E. coli* strains recovered from D24 differed in phylogroup (one isolate), virulence factors (all three isolates), and antimicrobial susceptibility (all three isolates). Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) suggested that all isolates from the uterine content of both dogs and the rectal swab of D23 were 100% the same, but different from all isolates in the rectal swab of D24. One isolate from the uterine content of each animal as well as rectal swabs were subjected to whole-genome sequencing (WGS). Both whole-genome multilocus sequence typing(wgMLST) and single-nucleotide polymorphism (SNP) analysis supported the hypothesis that the isolates from the uterine content of both animals and the rectal swab of D23 were clonal. Taken together, these clinical features, pathology, microbiology, and molecular findings suggest, to the best of our knowledge, the first transmission of *E. coli* associated with pyometra between two animals. These results could impact the management of sites where several females cohabit in the same local area such as kennels.

Keywords: ExPEC; EnPEC; UPEC

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Short Communication/Note

Pyometra is the most frequently observed reproductive disease in bitches, affecting up to 25% of unspayed females [1,2]. The disease is characterized by bacterial infection of the uterus with local and potentially fatal systemic clinical manifestations such as prostration, anorexia, purulent vulvar discharge, sepsis, and multi-organ dysfunction [3–5]. Despite its relevance, the pathogenesis of this disease remains poorly understood. It is believed that bacterial species may cause pyometra to ascend from the host's intestinal tract, causing a non-transmissible opportunistic infection [2,5,6]. In this study, we describe an in-depth investigation of the possible transmission of *Escherichia coli* associated with pyometra in two bitches.

A 5-year-old female Chow Chow (D23) was referred to the Veterinary Hospital of the Universidade Federal de Minas Gerais (VH-UFMG) with a purulent vulvar discharge. In addition to the vulvar discharge, the animal was hyperthermic (41 °C) and showed signs of prostration, anorexia, and diarrhea, suggestive of an open pyometra. The examinations also revealed anemia (hematocrit: 36%; RV: 37–55%), thrombocytopenia (platelets: 124,000/mm^2; RV: 175,000–500,000/mm^2, and azotemia (creatinine: 1.57 mg/dL; RV: 0.5–1.5 mg/dL). The animal underwent ovariohysterectomy (OHE) surgery. Just after the procedure, a sample from the uterine content and feces from the rectal ampulla of the dog were collected by needle aspiration and swab, respectively. The samples were refrigerated at 4 °C until processing for a maximum of 24 h. Samples from the uteri and ovaries were collected for histopathological analysis.

After 5 days, another female dog (D24) from the same litter and cohabiting with D23 was referred to VH-UFMG with similar symptoms including prostration, anorexia, and purulent vulvar discharge. These two dogs were the only animals in their household. Examination results indicated anemia (erythrocytes: 4.48 million/mm^2; RV: 5.5–8.5 million/mm^2 and hematocrit 25%), leukocytosis (leukocytes: 28,200 mm^2; RV: 6000–17,000 mm^2), thrombocytopenia (platelets: 90,000 mm^2), decreased blood urea nitrogen (BUN: 17.69 mg/dL; RV: 20–56 mg/dL), increased alkaline phosphatase (ALP: 157 U/L; RV: 40–156 U/L). This animal also underwent OHE surgery and again, uterine tissue, uterine content, and rectal swab samples were collected. After surgery, both animals were treated with amoxicillin/clavulanic acid and metronidazole.

Samples from the uteri and ovaries of both animals were fixed by immersion in 10% buffered formalin for 24 h, processed for paraffin embedding, and sectioned (3-µm thick), and stained with hematoxylin and eosin for histopathology. The uteri of both bitches were enlarged and filled with a significant amount of purulent brown material. Both animals were in diestrus, which was confirmed by the discovery of multiple corpora lutea. In addition, the ovaries of one bitch (D23) had neutrophilic arteritis and fibrinous thrombi, partially occluding the artery.

Microscopically, in both animals, the uterine lumen was filled with many neutrophils, fibrin, bacterial aggregates, and, in D23, also blood. There was a severe diffuse neutrophilic and lympho-histioplasmacytic endometrial inflammatory infiltrate, with marked neutrophilic exocytosis into the uterine lumen and endometrial glands. In D23, there was severe multifocal necrosis with endometrial ulceration extending to the superficial endometrial glands, with intense endometrial hemorrhage, fibrin deposition, and moderate fibroplasia (Figure 1); many other endometrial veins were filled with fibrin thrombi, which partially occluded the lumen. In D24, there was mild multifocal endometrial ulceration. These concurrent findings are highly indicative of the histopathological lesions observed in cases with severe pyometra [7,8]. Endometrial necrosis and ulceration may also be observed in pyometra cases; however, which determines the intensity of necrosis in each case is not well-defined [8]. In D23, the remaining endometrial epithelial cells and in D24, epithelial cells of the luminal endometrial epithelium and superficial endometrial glands were columnar with finely vacuolated cytoplasm, morphologically indicative of decidual reaction. In both animals, endometrial glands were diffusely and markedly ecstatic with the accumulation of neutrophils and mucous [8] (Figure 1). In addition, a multifocal moderate histioplasmacytic and neutrophilic inflammatory infiltrate extended into the myometrium. Therefore, a morphological diagnosis of pseudoplacentational endometrial hyperplasia and pyometra was established in both bitches [7]. Interestingly, as observed in these bitches, a recent study described the high frequency of the association of pseudoplacentational endometrial hyperplasia with pyometra in female dogs [9].

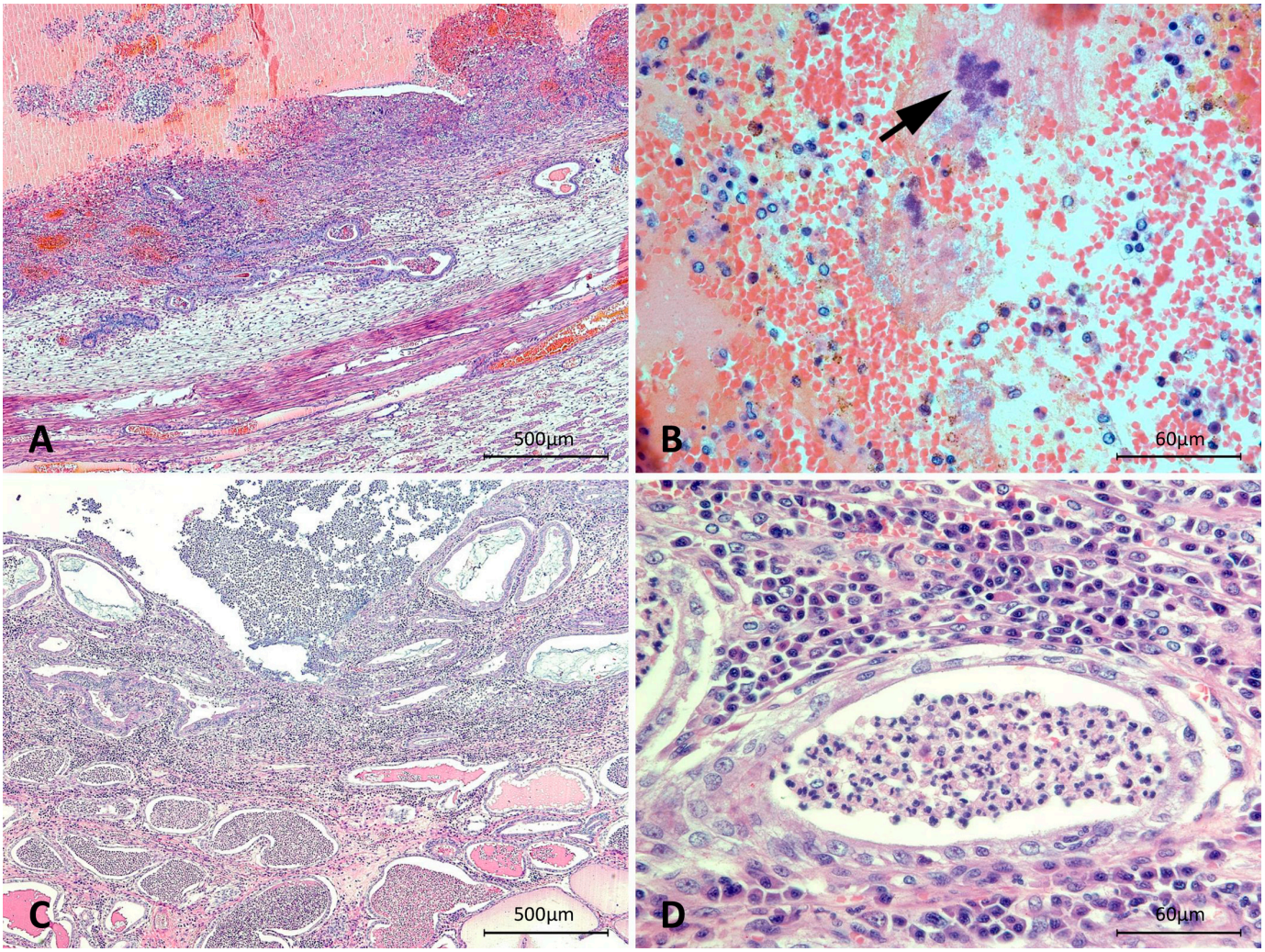

Figure 1. Canine uteri. (**A**) D23—uterine lumen filled with large amounts of blood and increased cellularity in the endometrium, with severe necrosis, endometrial ulceration, and hemorrhage. Endometrial glands are markedly ecstatic. (**B**) D23—higher magnification of A: hemorrhagic uterine luminal content, with neutrophils, plasma cells, fibrin, and bacterial aggregates (arrow). (**C**) D24—severe diffuse endometritis, with endometrial glands markedly ecstatic with an accumulation of inflammatory cells and mucous, and superficial luminal and glandular epithelium composed of columnar and finely vacuolated cells. (**D**) D24—higher magnification of C: severe diffuse interstitial lympho-histioplasmacytic infiltrate, and an endometrial gland filled with neutrophils. Hematoxylin and eosin, scale bars = 500 µm (**A**,**C**), 60 µm (**B**,**D**).

The uterine contents and rectal swabs were plated on Mueller–Hinton agar (Sparks, BD, USA) supplemented with 5% equine blood and in MacConkey agar (Kasvi, São José dos Pinhais, Brazil), followed by aerobic and anaerobic incubation at 37 °C for 48 h. Twelve lactose-fermenting colonies from each sample (48 isolates in total) were subjected to a species-specific polymerase chain reaction (PCR) to identify *E. coli* [10]. Thus, the isolates were subjected to PCR to determine *E. coli* phylogroups [11] and to detect the main virulence genes associated with the extraintestinal pathogenic *E. coli* (ExPEC) pathotype, namely, fimbriae type I (*fimH*), fimbriae type I central region (*focG*), fimbriae type P (*papC* and *papG* alleles II and III), fimbriae type S (*sfaS*), cytotoxic necrotizing factor type 1 (*cnf1*), α-hemolysin (*hlyA*), uropathogenic specific protein (*usp*), aerobactin (*iutA*), and serum resistance (*traT*) [12,13]. Additionally, antimicrobial susceptibility to amoxicillin/clavulanic acid, ampicillin, ceftiofur, ciprofloxacin, enrofloxacin, neomycin, gentamicin, doxycycline,

oxytetracycline, and trimethoprim/sulfamethoxazole was assessed using the disk diffusion method and interpreted according to the Clinical and Laboratory Standards Institute [14,15].

All *E. coli* strains isolated from the uterine contents of both female dogs were classified into the same phylogroup (B2) (Figure 2). This result was not surprising as *E. coli* is the most common bacterial organism found in a pyometra, and these isolates tend to cluster mainly in phylogroup B2 [5,16,17]. Furthermore, the uterine *E. coli* isolates also had the same ExPEC virulence factor-encoding genes (*fimH*, *focG*, *papC*, *papG*, *cnf1*, *hlyA*, and *usp*) (Figure 2), virulence factors also commonly associated with endometrial pathogenic *E. coli* [16,18]. These virulence factors, mainly the fimbriae-encoding genes (*fimH*, *focG*, *papC* and *papG*), are of great relevance for the establishment of *E. coli* infections in the canine uterus [13,16,19]. Additionally, *E. coli* strains from the uterine contents had no antimicrobial resistance (Figure 2).

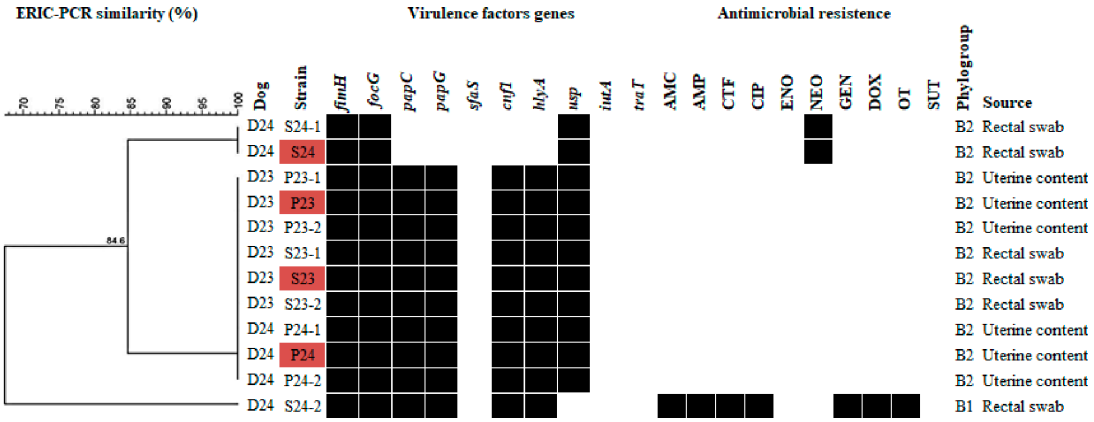

Figure 2. Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) similarity, virulence factors, phylogroup, and antimicrobial profile of *E. coli* isolated from rectal swabs and uterine contents of two cohabiting bitches (D23 and D24). Legend: P: uterine content; S: rectal swab, AMC: amoxicillin/clavulanic acid, AMP, ampicillin, CTF: ceftiofur, CIP: ciprofloxacin, ENO: enrofloxacin, NEO: neomycin, GEN: gentamicin, DOX: doxycycline, OT: oxytetracycline, and SUT: trimethoprim/sulfamethoxazole. Isolates marked in red were selected for whole genome sequencing.

Interestingly, the *E. coli* isolates recovered from the D23 rectal swab showed the same phylogroup, virulence factors, and antimicrobial profile as the isolates recovered from the uterus of D23 and D24. In contrast, the strains isolated from the D24 rectal swab differed in phylogroup (four isolates), virulence factors (all twelve isolates), and antimicrobial susceptibility (all twelve isolates). Based on these results, two hypotheses were raised: first, that the *E. coli* strain colonizing D23′s rectum ascended to the uterus of this animal, causing the infection; second, that this *E. coli* strain was transmitted to D24, causing pyometra.

To further investigate the possible clonal origin of the isolates, six *E. coli* strains from each dog (tree per isolation) were fingerprinted by Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) [20,21] and random repetitive extragenic palindromic (REP)-PCR [22,23]. These techniques were successfully used in previous investigations of outbreaks caused by Enterobacteriaceae [24]. Furthermore, both ERIC-PCR and REP-PCR suggested that all isolates from the uterine content of both dogs and the rectal swab of D23 were 100% similar, but different from all isolates from the rectal swab of D24 (similarity $\leq$84.6%) (Figure 2), reinforcing the suspicion that *E. coli* was transmitted from D23 to D24, causing pyometra.

To better evaluate the hypothesis of transmission of *E. coli* associated with pyometra between the two bitches, four strains were subjected to whole-genome sequencing, currently considered the technique with the highest accuracy and resolution for these cases [25]. One isolate from each site (uterus and rectum) from each animal was included (marked in red in Figure 2). Genomic deoxyribonucleic acid was extracted using the Maxwell 16® Research Instrument (Promega, Madison, WI, USA) combined with lysozyme (10 mg/mL)

and proteinase K (20 mg/mL). The genome was sequenced using the Ion Torrent PGMTM in a mate-pair sequencing kit with an insert size of 3 kbp (~144-fold coverage) and with a fragment sequencing 400 bp kit (~318-fold coverage). The quality of the raw data was analyzed using FastQC (Babraham Bioinformatics), trimming of reads to remove adapters and 3′ ends with Phred's quality score <20 was conducted with an in-house-script (available at https://github.com/aquacen/fast_sample), and assembly was performed using SPAdes 3.5.0 [26]. With default parameters, automatic annotation was performed using Prokka 1.10 (Rapid Bacterial Genome Annotation) software [27]. VirulenceFinder 2.0, ResFinder 4.1, and SerotypeFinder 2.0 [28–33] were used to identify virulence factors, antimicrobial resistance genes, and mutations, and to predict the O serotype. Multilocus sequence typing (MLST) 2.0 was used to determine sequencing types (ST) according to the Achtman MLST scheme [28,34–36] and Phyloviz v 2.0, using the goeBURST algorithm [37,38], was used to infer the population structure with clonal complexes (CCs) composed of all strains sharing at least six identical alleles (single-locus variant). Whole-genome MLST of the four isolates was performed using Ridom SeqSphere+ 4.1.9 [39] and 13 reference *E. coli* strains from previous studies on humans were included for comparison purposes. These strains were subjected to single-nucleotide polymorphism (SNP) analysis using CLC Workbench software v 6 (Qiagen, Aarhus, Denmark). Parameters for alignment were settled as mismatch cost = 2; insertion/deletion cost = 1. Parameters to SNP calls were defined to minimum coverage = 10; minimum variant frequency = 50%; filter 454/ion homopolymer indels = 1. Other parameters for alignment and SNP calls remained as the default.

The three likely clonal isolates were classified into a new sequence type on the same CC of ST372 (Figure 3; Table 1), uropathogenic *E. coli* previously described in several reports on dogs and humans, commonly causing genitourinary infection [40–45]. Furthermore, the SNP analysis confirmed the clonal relationship between the isolates from the uterine content of D23 and D24 and the strains isolated from the rectum of D23, with a maximum difference of 22 SNPs.

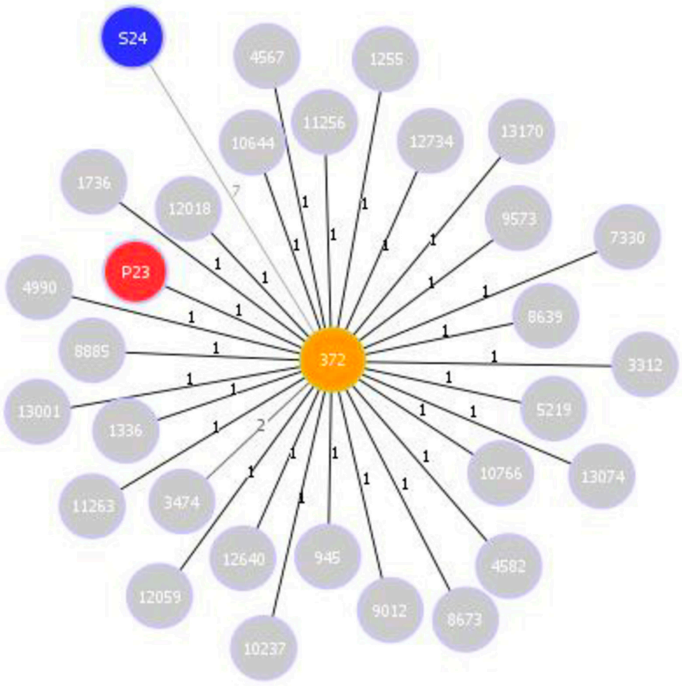

Figure 3. goeBURST population snapshot of clonal complex (CC) 372. Only isolates with a single locus variance (SLV) and isolates from the present study were included: the S24 isolate is marked in blue; ST372 (founder) is marked in orange, P23 is marked in red, and other isolates obtained from the public *E. coli* MLST database are marked in gray; line numbers indicate allelic variance.

Table 1. Results of virulence factors, resistance gene detection, and multilocus sequence typing (MLST) of the four *E. coli* isolates recovered from the uterine contents and rectal swabs of two cohabiting Chow Chow bitches.

Animal	Source	Sample ID	Accession Number	O Serotype [1]	Antimicrobial Resistance Genes	Virulence Factors
D23	Uterine content	P23	JAMJIL000000000	O4:H31	*mdf(A) sitABCD*	*papC*, *cnf1*, *focI*, *hra*, *papA_F13*, *focG*, *usp*, *chuA*, *cea*, *clbB*, *focCsfaE*, *fyuA*, *ibeA*, *iroN*, *irp2*, *iss*, *mchB*, *mchC*, *mchF*, *mcmA*, *ompT*, *sitA*, *terC*, *vat*, and *yfcV*
	Rectal swab	S23	JAMJIK000000000			
D24	Uterine content	P24	JAMJIJ000000000			
	Rectal swab	S24	JAMJII000000000	O5:H22	*mdf(A) sitABCD*	*gad*, *kpsE*, *kpsMII*, *pic*, *sfaD*, *tcpC*, *focG*, *usp*, *chuA*, *cea*, *clbB*, *focCsfaE*, *fyuA*, *ibeA*, *iroN*, *irp2*, *iss*, *mchB*, *mchC*, *mchF*, *mcmA*, *ompT*, *sitA*, *terC*, *vat*, and *yfcV*

[1] Predicted serotype by SerotypeFinder 2.0 [29,33].

Moreover, as expected, the isolate from the rectum of D24 differed greatly from these three isolates (Figure 4). Together, these results confirm that the strain colonizing the rectum of D23 ascended to the uterus of this bitch, causing pyometra. This is the currently accepted pathogenesis of this disease [2]. On the other hand, this same bacterium was later transmitted to the uterus of D24, causing pyometra. This is the first report of a likely transmission of *E. coli* pyometra between two female dogs.

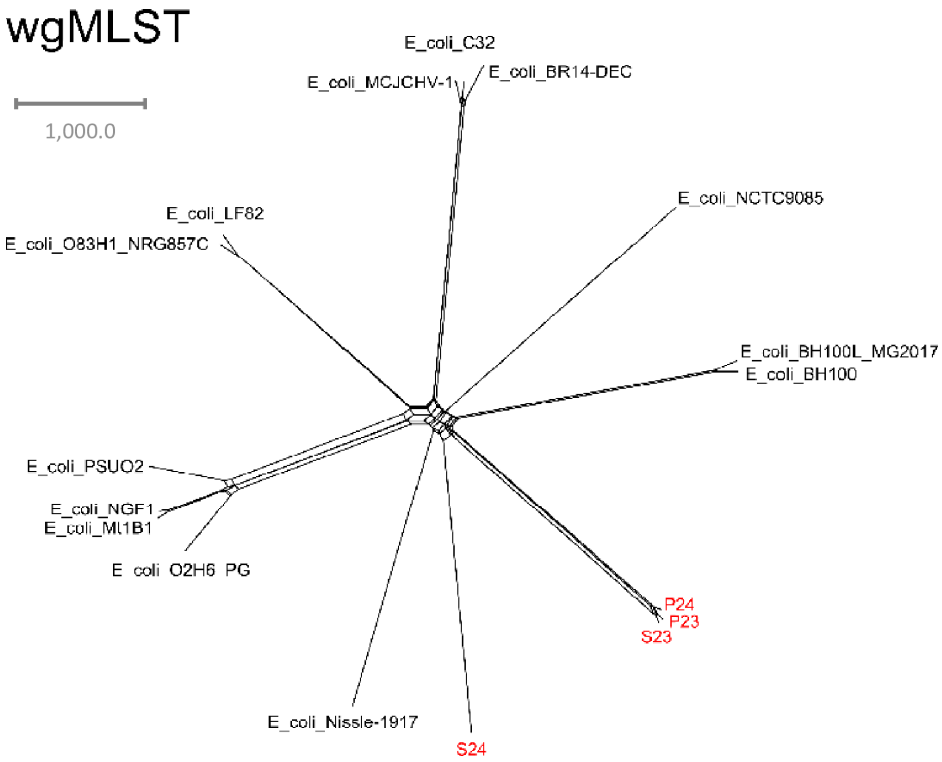

Figure 4. wgMLST phylogeny tree including the present study isolates (in red) and reference strains added for comparison purposes.

Despite its clinical relevance, the pathogenesis of canine pyometra remains poorly understood. So far, pyometra has been recognized as a non-transmissible infectious disease [1,2,46]. The present study describes a likely transmission of *E. coli* pyometra between two dogs. This hypothesis was first raised after two cohabiting bitches from the same litter developed pyometra in a short period. The genetic similarity seen in the genome of these three strains confirmed the clonal origin, reinforcing the hypothesis of transmission. Although it is impossible to determine the source of contamination for D24, the source was direct or indirect contact with feces or vulvar discharge from D23. Previous studies have

suggested that, in addition to feces, uterine contents may be a source of dissemination of pathogenic *E. coli* to the environment, possibly contaminating other hosts [13,19,47]. It is worth mentioning that D23 had an open pyometra, and according to the owner, a constant purulent discharge had been observed.

A noteworthy point is that the isolate causing pyometra in both dogs was classified in the same CC of ST372, a well-known pathogen for dogs and humans. In addition, several studies with ExPEC isolated from dogs have shown a high similarity of these isolates with those recovered from humans cohabiting in the same environment [48–51].

The clone that caused pyometra in both dogs was also isolated from the gut microbiota of dog 23 (D23), but not from the gut of dog 24 (D24). However, it is not possible to completely rule out that this clone was present in the gut of D24. Furthermore, it is also possible that both dogs acquired this clone from an unknown common source. Importantly, these two were the only dogs sharing their environment. Finally, it is also important to consider that some breeds seem to be at increased risk of developing pyometra including Chow Chow [52–54].

For the first time, this study describes the possible transmission of *E. coli* pyometra in dogs. Our findings suggest that in sites with more than one unspayed female, animals with suspected or confirmed pyometra should be isolated from other bitches until clinical resolution. This finding will be relevant to kennel managers, owners with several dogs, and even hospitals.

Author Contributions: All authors contributed to the study conception and design. Material preparation and samples collection, P.H.S.d.S.; Laboratory analysis, R.G.C.X., C.H.S., F.F.A., and F.L.P.; First draft of the manuscript, R.G.C.X., C.H.S., P.H.S.d.S., F.F.A., F.L.P., H.C.P.F., P.M.C.F., R.L.S., and R.O.S.S. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The whole-genome shotgun project was deposited in GenBank/NCBI under the BioProject accession number PRJNA824036, Biosample SAMN27382112 (P23), SAMN27382113 (S23), SAMN27382114 (P24), and SAMN27382115 (S24). Genome accession numbers: JAMJIL000000000 (P23), JAMJIK000000000 (S23), JAMJIJ000000000 (P24), and JAMJII000000000 (S24) [https://www.ncbi.nlm.nih.gov/bioproject/PRJNA824036/, accessed on 8 September 2022].

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Conflicts of Interest: The authors declare no conflict of interest.

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Association between bacterial pathogenicity, endometrial histological changes and clinical prognosis in canine pyometra

Rafael Gariglio Clark Xavier, Clarissa Helena Santana, Paloma Helena Sanches da Silva, Amanda Oliveira Paraguassú, Rafael Romero Nicolino, Patrícia Maria Coletto Freitas, Renato de Lima Santos, Rodrigo Otávio Silveira Silva *

Escola de Veterinária, Universidade Federal de Minas Gerais, Antônio Carlos Avenue, 6627, Belo Horizonte, MG, 31.270-901, Brazil

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ABSTRACT

Despite the high frequency and clinical relevance of canine pyometra, its pathogenesis remains poorly understood. In this study, the clinical data, histopathological alterations, and microbiological findings of 39 dogs with pyometra were analyzed to assess possible associations. The mean age of the affected animals was 9.6 ± 3.8 years; 76.3 % (29/38) had open cervix pyometra, 88 % (22/25) had tachypnea, 71 % (27/38) had anorexia, and 60.5 % (23/38) had leukocytosis. Histopathological analysis revealed that 66.5 % (26/39) of the uteri had a high degree of inflammation (score 4). Third-degree hyperplasia of the endometrial epithelium (72 %, 28/39) and intralesional or intrauterine bacteria (66.5 %, 26/39) were identified in most animals. Bacterial isolates were obtained from 82 % (32/39) of the uterine contents and five bacterial species were identified. *Escherichia coli*, classified in phylogroup B2, is associated with virulent adhesion genes (*fimH*, *focG*, and *papC*), and serum resistance (*traT*) was the most common isolate. There was an association between the detection of *papC* in *E. coli* isolates and higher necrosis scores. Additionally, the necrosis score was positively associated with the length of hospitalization, with each point increase in the necrosis score leading to two more days of hospitalization. These results suggest that *papC*-positive *E. coli* play an important role in the severity of pyometra in dogs. The present study revealed the possibility of using this virulence gene to better understand the prognosis of the disease in an affected animal.

1. Introduction

Pyometra is the most common reproductive disease in dogs [1] and is characterized by a suppurative infection with the accumulation of purulent exudates in the uterus [2,3]. Despite its high prevalence and relevance as a life-threatening disease, the pathogenesis of pyometra remains poorly understood. Studies have suggested factors that predispose patients to the occurrence of the disease, such as age greater than 8 years [4,5]. In addition, some breeds seem more predisposed, such as Boxer, Chow Chow, Cocker Spaniel, Collie, Golden Retriever, Labrador Retriever, Pinscher, Rottweiler, Saint Bernard, and Schnauzer [6]. In addition, dogs with pseudoplacentational endometrial hyperplasia appear to be more predisposed [7].

Among the etiological bacteria involved, extraintestinal pathogenic *Escherichia coli* (ExPEC) is by far the most common pathogen isolated in canine pyometra and has been reported in 57%–100 % of cases [8–10]. These isolates are phylogenetically and epidemiologically distinct from *E. coli* strains found in the intestine as commensals or those that cause gastrointestinal disorders [10–12]. In canine pyometra, *E. coli* strains found in the uterine contents are commonly associated with a specific phylogroup (B2) and have several virulence factors that enhance the colonization of extraintestinal sites, including mostly adhesins and toxins [10,13,14]. These virulence factors are believed to play key roles in the development of canine pyometra [10,13,15]. However, it is largely unknown how these ExPEC virulence traits are linked to lesions and clinical severity of pyometra. Therefore, the objective of this study was to verify the associations among clinical data, different degrees of endometrial lesions, and bacterial pathogenicity in female dogs with pyometra.

* Corresponding author.
E-mail address: rodrigo.otaviosilva@gmail.com (R.O.S. Silva).

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2. Materials and methods

2.1. Animals

A total of 39 female dogs that underwent ovariohysterectomy surgery at the Veterinary Hospital of the Universidade Federal de Minas Gerais (VH-UFMG) were included in this study. Immediately after surgery, the purulent uterine content was sampled, and uterine tissue samples were collected for histopathology. This study was approved by the Ethics Committee on Animal Use of UFMG (Protocol No. 51/2015).

2.2. Clinical and epidemiological data

The following data were obtained from the medical clinical records analyzed during the medical consultation, when available: breed, age, type of pyometra, previous use of exogenous progesterone, rectal temperature, respiratory frequency, anorexia, occurrence of vomiting and diarrhea, hemogram, leukogram, renal function, antimicrobial treatment, length of hospitalization, total time of hospitalization, and outcome (Table 1).

Table 1
Clinical and laboratory variables of the female dogs with pyometra.

Variable	Total
Age (years: n=39)	
Mean [Mín.–Máx. ±SD]	9.6 [3–20 ± 3,8]
Previous use of exogenous progesterone (n=34)	
Yes – n (%)	4 (11.7)
Breed (n=39	
Mixed-breed – n (%)	6 (15.6)
Yorkshire Terrier – n (%)	5 (13)
Poodle – n (%)	4 (10.4)
Golden Retriever – n (%)	3 (7.8)
Labrador Retriever – n (%)	3 (7.8)
Pinscher – n (%)	3 (7.8)
Rottweilers – n (%)	3 (7.8)
Others[a] (%)	12 (31.2)
Pyometra (n=38)	
Open cervix – n (%)	29 (76.3)
Closed cervix – n (%)	9 (23.7)
Hyperthermia (RV[c]: 37.2–39°C: n=35)	
Yes – n (%)	3 (8.5)
Tachycardia (n=32)	
Yes – n (%)	14 (43.7)
Tachypnea (n=25)	
Yes – n (%)	22 (88)
Anorexia (n=38)	
Yes – n (%)	27 (71)
Vomit (n=38)	
Yes – n (%)	9 (23.6)
Diarrhea (n=38)	
Yes – n (%)	10 (26.3)
Anemia (hematocrit: RV: 37–55 %: n=38)	
Yes – n (%)	16 (42.1)
Leukocytosis (cells: RV: 6000–17,000 mm^2: n=38)	
Yes – n (%)	23 (60.5)
Azotemia (creatinine: RV: 0.5–1.5 mg/dL: n=38)	
Yes – n (%)	13 (44.8)
Antimicrobial treatment (antibiotic: n=38)	
Amoxicillin/Clavulanic Acid/Metronidazole – n (%)	20 (52.5)
Amoxicillin/Clavulanic Acid – n (%)	7 (18.5)
Cephalexin – n (%)	4 (10.5)
Cephalexin/Metronidazole – n (%)	3 (7.5)
Others[b]	4 (10)
Length of hospitalization (days: n=39)	
Mean [Min.–Max.]	2.4 [0–7]
Death (n=34)	
Yes – n (%)	1 (2.6)

[a] Other breeds: American Pit Bull Terrier, Chow Chow, German Shepherd, Lhasa Apso, Border Collie, Maltese, Pekingese, Shih Tzu.
[b] Other antimicrobial treatments included cephalexin/clindamycin/enrofloxacin, clindamycin, doxycycline/enrofloxacin, and doxycycline.
[c] RV: reference values.

2.3. Uterine histopathological analyses

Uterine samples were fixed in 10 % neutral buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin (H&E) [16], followed by microscopic evaluation. Based on a previously described method [7,17], each sample received semi-quantitative scores according to lesion characteristics and intensity for the following lesions: degree of necrosis (score 0 to 3), inflammation (score 0 to 4), hyperplastic changes (endometrial gland ectasia [CEH] (score 0 to 3), pseudoplacentational hyperplasia of the endometrial epithelium [PEH] (score 0 to 3)), and qualitative evaluation for bacterial presence. Details of the parameters used in the present study are provided in Supplementary Table 1.

2.4. Bacterial isolation and identification

The uterine contents were streaked on two plates with Mueller-Hinton agar (Kasvi, Italy) supplemented with equine blood (5 %) and one plate with MacConkey agar (Difco, USA). The plates were incubated at 37 °C for 48 h under aerobic and anaerobic conditions. Isolates identified as *E. coli* were subjected to species-specific polymerase chain reaction (PCR) to confirm its identity [18]. The identities of the other isolates were confirmed by matrix-assisted laser desorption ionization-time of flight (MALDI-ToF) mass spectrometry (Bruker Daltonics, Bremen, Germany), as previously described [19,20]. Briefly, for each isolate, approximately 1 μL of formic acid (70 %) and 1 μL of a saturated solution of α-cyano-4-hydroxycinnamic acid were applied to the spot and allowed to air dry. Spectra were acquired using a FlexControl MicroFlex LT mass spectrometer with a 60-Hz nitrogen laser and approximately 240 laser shots. Parameters for mass range detection were defined as follows: ion source 1 voltage was 19.99 kv, ion source 2 voltage was 18.24 kv, and lens voltage was 6.0 kv for data acquisition, allowing the identification from 1960 to 20,137 *m/z*. *E. coli* DH5 alpha was used for calibration and scores ≥2.3 were used for a species-level identification as recommended by the manufacturer.

2.5. Characterization and virulence genotyping of E. coli isolates

E. coli isolates were subjected to previously described PCRs to determinate the phylogroup (A, B1, B2, C, D, E, F, or clade I) [21] and virulence factors corresponding to the ExPEC pathotype: fimbriae type P (*papC* and *papG* allele II and III), fimbriae type I (*fimH*), fimbriae type I central region (*focG*), fimbriae type S (*sfaS*), cytotoxic necrotizing factor type 1 (*cnf1*), uropathogenic specific protein (*usp*), α-hemolysin (*hlyA*), aerobactin (*iutA*), and serum resistance (*traT*) [22,23].

2.6. Statistical analysis

The results were analyzed using EngineRoom software [24]. To analyze the association between bacterial species, *E. coli* characteristics, histopathological analyses, and clinical data, a multiple-proportion comparison test was performed. This test is based on a chi-square distribution and a pooled estimate of the population proportion to estimate the standard error of the test statistic. If a significant difference was found in the overall test, a pairwise comparison method with the Marascuilo Procedure was used to identify the specific pairs of proportions that differed significantly. Statistical significance of the results was set at $p \leq 0.05$ for the analyzed characteristics [25].

3. Results

3.1. Clinical metadata

The availability of clinical information varied between the groups (Table 1). The age of the animals ranged from 3 to 20 years (mean and standard deviation 9.6 ± 3.8 years) and most clinical presentation of

pyometra were open cervix (76.3 %). Previous use of exogenous progesterone was confirmed in four (11.7 %) animals. Most animals had tachypnea (88 %), anorexia (71 %), or leukocytosis (60.5 %). The median length of hospitalization was 2.4 days (range, 0–7 days) and only one death was recorded (2.6 %).

3.2. Classification of the endometrial histopathological lesions

Histopathological analysis (Table 2; Figs. 1 and 2) revealed that most uteri (26/39, 66.5 %) had a high degree of inflammation (score 4). A score 3 of a decidual reaction and hyperplasia of the endometrial epithelium and superficial endometrial glands with moderate to severe ectasia of the deep endometrial glands and intralesional bacteria were identified in most animals (72 % and 66.5 %, respectively), and 41 % (16/39) of the uteruses had no necrosis (score 0). Endometrial hemorrhage was identified in 46.1 % (18/39) of the uteri, with intensities ranging from mild (11.1 %, 2/18) to moderate (33.3 %, 6/18) to severe (25.6 %, 10/18) (Fig. 2).

3.3. Bacterial isolates, phylogroup, and virulence factors of E. coli isolates

Bacterial isolates were cultured from 82 % (32/39) of the intrauterine contents. Of these 32 culture-positive samples, five different bacterial species were identified (Table 3), with *E. coli* 75 % (24/32) being significantly the most frequently isolated species ($p = 0.0001$). No mixed bacterial infections were identified.

3.4. E. coli phylogroup and virulence genes

Three phylogroups were identified among the *E. coli* isolates (Table 4). A total of 83.3 % (20/24) of the isolates were classified into phylogroup B2, which was the most frequent ($p < 0.00001$). Phylogroups A, C, D, and E were not identified in this study.

All tested virulence genes were detected in *E. coli* isolates from intrauterine contents at different frequencies (Table 5). Virulence genes associated with adhesion (*fimH*, 100 %; *focG*, 66.5 %; *papC*, 50 %) and serum resistance (*traT*, 62.5 %) were detected in most *E. coli* isolates.

Table 2
Lesion degrees based on the histopathological analyses: inflammation, necrosis, hyperplastic changes, and bacterial presence endometrial in uterus samples of canine pyometra.

Score	Total cases (%)
Inflammation	
0	2 (5)
1	1 (2.5)
2	1 (2.5)
3	9 (23)
4	26 (66.5)
Necrosis	
0	16 (41)
1	10 (25)
2	10 (25)
3	3 (7.5)
Cystic endometrial hyperplasia	
0	36 (92.5)
1	3 (7.5)
2	0
3	0
Pseudo-placentational endometrial hyperplasia	
0	8 (20)
1	3 (7.5)
2	0
3	28 (72)
Intralesional/intrauterine bacteria	
0	13 (33.5)
1	16 (41)
2	10 (25.5)
Total	**39 (100)**

3.5. Associations between clinical, histological, and microbial findings

An association between the isolation of *E. coli* positive for the adhesin-encoding gene *papC* and a higher degree of uterine necrosis was observed ($p = 0.03$). In addition, the degree of necrosis was associated with the length of hospitalization ($p < 0.034$): each increase in the degree of uterine necrosis represented an increase in two days of hospitalization, suggesting that higher degrees of necrosis corresponded to more severe clinical manifestations in the dogs, resulting in a longer hospital stay. No other associations between the clinical, histological, and bacterial findings were observed in the present study.

4. Discussion

Pyometra is the most frequent reproductive disease in dogs. Despite its known relevance, there are very few studies comparing the clinical, pathological, and microbiological data of affected animals, which can lead to a better understanding of the pathogenesis of this disease. Our results suggest that *E. coli* positivity for a specific fimbria (*papC*) can directly affect uterine necrosis, increasing the hospitalization of the affected animal.

In this study, the mean age of the sampled female dogs (approximately 9 years) was similar to that of previous studies, reinforcing the hypothesis that dogs older than eight years have a higher risk of pyometra [26]. Although the effect of age is not fully understood, several hypotheses have been proposed, including immunosenescence, reduction in the capacity of defeat infections, and repeated estrous cycles over the years, which would repeatedly cause elevated estrogen levels, leading to endometrial proliferation [5,27,28]. The use of steroid hormones to prevent pregnancy is also a known predisposing factor [29–31]. In the present work, 1 in 10 owners confirmed the use of this medication, a frequency lower than that reported in other studies [32,33]. However, it is not possible to state that all veterinarians questioned the owners regarding their previous use of exogenous progesterone during anamnesis, which may have influenced the frequency.

The most common clinical signs of pyometra observed in the present study were purulent vulvar discharge (open cervical pyometra), tachypnea, and anorexia, which were present in more than 71 % of the animals, similar to the findings of other studies [9,34,35]. Other clinical signs, including hyperthermia, tachycardia, vomiting, and diarrhea, were found at different rates (between 8.5 % and 88 %) and reflected the systemic involvement of the disease [28,31,36].

Leukocytosis (60.3 %), azotemia (44.8 %), and anemia (42.1 %) were also frequently observed in affected dogs, corroborating previous studies [9,34,37,38]. Anemia occurs due to uterine hemorrhage, which is commonly associated with inflammatory exudates in cases of pyometra. Uterine hemorrhage was diagnosed in 46.1 % of the dogs sampled in this study. In addition, suppression of the bone marrow by endotoxins produced by gram-negative bacteria, mainly *E. coli*, can also aggravate anemia. Endotoxemia is also responsible for the intense leukocytosis typically seen in these cases [39,40] and can also contribute to kidney damage together with immune complex deposition in the renal glomeruli, leading to azotemia and possible multiple organs dysfunction [1,38,40].

The length of hospitalization, which is commonly used as a nonspecific indicator of the severity of many diseases [37], was quite low (2.4 days). Previous studies have suggested that up to two days of hospitalization is usually sufficient in uncomplicated cases, suggesting that most dogs included in the present study fall into this classification [9,37]. Corroborating these assumptions, the lethality rate (2.6 %) was also low in comparison to previous studies (up to 20 %) [9,34]. One hypothesis for the low severity of the cases included in this study was the high proportion of open cervical pyometra (76.3 %). It is known that the drainage of uterine reduce the risk of complications, including life-threatening conditions like septicemia [35,39].

High scores (scores 3 and 4) for inflammation (89.5 %) were

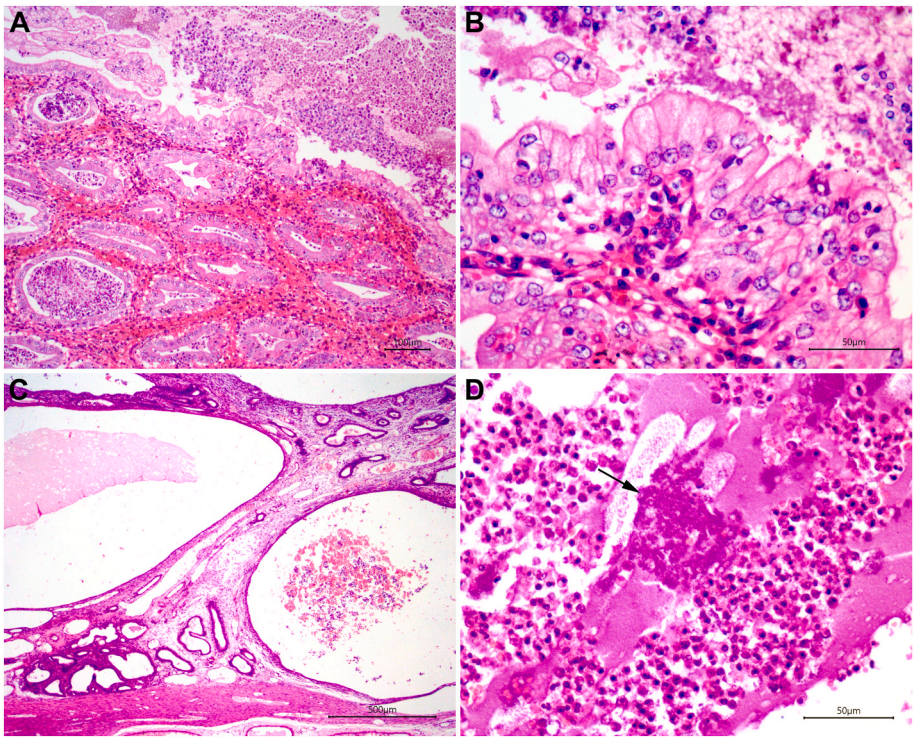

Fig. 1. Canine pyometra. (A) Uterus with severe inflammation (score 4) and pseudoplacentational endometrial hyperplasia (PEH) (score 3). Inflammation is composed of interstitial and luminal infiltration of large numbers of neutrophils, with cellular debri, endometrial hemorrhage, and ectasia of endometrial glands. H&E, scale bar = 100 µm. (B) Uterus with severe inflammation (score 4) and PEH (score 3), with a columnar and finely vacuolized luminal epithelium. H&E, scale bar = 50 µm. (C) Uterus with marked inflammation (score 3) and cystic endometrial hyperplasia (CEH) (score 3), characterized by severe glandular ectasia. H&E, scale bar = 500 µm. (D) Uterine luminal contents with bacterial aggregates (arrow) and large numbers of neutrophils and some erythrocytes. H&E, scale bar = 50 µm.

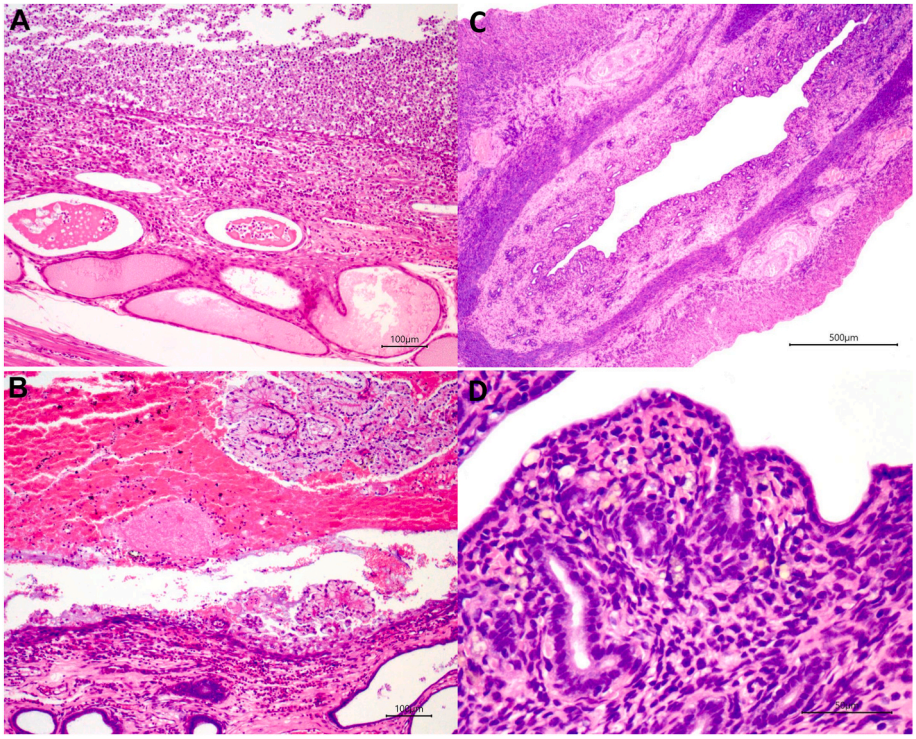

Fig. 2. Canine pyometra (A and B) and canine uterus (C and D). (A) Uterus with severe inflammation (score 4) and necrosis (score 3), characterized by loss of some superficial endometrial glands and complete loss of endometrial epithelium. H&E, scale bar = 100 µm. (B) Uterus with marked inflammation (score 3), PEH (score 3), and severe intraluminal hemorrhage with fibrin deposition. H&E, scale bar = 100 µm. (C) Uterus with an empty lumina. Endometrium, myometrium and perimetrium with normal thickness and superficial and deep glands with normal diameter. H&E, scale bar = 500 µm. (D) Endometrium with cuboidal luminal epithelium, superficial and deep glands with normal diameter lined by a cuboidal epithelium. Lamina propria with normal connective tissue. H&E, scale bar = 50 µm.

Table 3
Bacterial species isolated from the intrauterine content of dogs with pyometra.

Organism	Total cases (%)
Escherichia coli	24 (61.5)
Streptococcus canis	3 (7.5)
Enterobacter cloacae	2 (5)
Proteus mirabilis	2 (5)
Klebsiella pneumoniae	1 (2.5)
No growth	7 (17.5)
Total	**39 (100)**

Table 4
Number of isolates and frequency of *Escherichia coli* phylogroups identified in the intrauterine content of dogs with pyometra.

Phylogroup	Total cases (%)
B2	20 (83.2)
B1	1 (4.2)
F	1 (4.2)
Not classified[a]	2 (8.4)
Total	**24 (100)**

[a] Identified as *E. coli* but not corresponding to any of the phylogroups [21].

Table 5
Number of isolates and frequency of *Escherichia coli* virulence genes identified in the intrauterine content of dogs with pyometra.

Virulence factors	Total cases (%)
Adhesion	
Fimbriae type I (*fimH*)	24 (100)
Fimbriae type I central region (*focG*)	16 (66.5)
Fimbriae type P (*papC*)	12 (50)
Fimbriae type P (*papG* allele II and III)	8 (33)
Fimbriae type S (*sfaS*)	7 (29)
Toxins	
α-hemolysin (*hlyA*)	11 (46)
Cytotoxic necrotizing factor type 1 (*cnf1*)	9 (37.5)
Uropathogenic specific protein (*usp*)	9 (37.5)
Iron acquisition	
Aerobactin (*iutA*)	11 (46)
Serum resistance	
Serum resistance (*traT*)	15 (62.5)
Total	**24 (100)**

observed in the histopathological analyses, and hyperplastic lesions were present in many female dogs (87 %), similar to the findings of a recently published study [41]. However, since CEH and PEH are two different hyperplastic conditions [42], these lesions were separated, and PEH (72 %) was more frequent than CEH (7.5 %) in female dogs with pyometra. These results are consistent with a previous study [7] and supports the notion that instead of which was proposed [43], pyometra is more frequently associated to PEH than CEH. Endometrial necrosis observed to varying degrees (57.5 %) can be caused by bacterial toxins, in addition to the neutrophilic inflammatory response [9].

As expected, *E. coli* was isolated from most of the uterine contents of the dogs with pyometra (61.6 %). This result is consistent with previous studies showing that *E. coli* is the main bacterium involved in pyometra [9,10,40,44]. To better understand the molecular characteristics of *E. coli* involved in pyometra, all isolates recovered in the present study were subjected to phylogroup identification and virulence factor detection, which have been widely used to better understand the colonization dynamics of this bacteria [21,45,46]. In the present study, B2 was the most frequent phylogroup, accounting for 83.2 % of *E. coli* isolates. This frequency is similar to that reported in previous studies on canine pyometra, suggesting a high capacity of phylogroup B2 strains to colonize the uterus [10,15,41,47].

Another interesting aspect of *E. coli* is the presence of virulence factors that enable infection of different tissues and sites [22,23]. Virulence factors that promote adhesion and colonization, particularly fimbriae, are considered of great relevance in the establishment of *E. coli* infections in the canine uterus [48–51]. In the present study, three adhesin-encoding virulence genes (*fimH*, *focG*, and *papC*) were the most commonly detected virulence factors, corroborating previous works [10,13,15,41]. Interestingly, the present study revealed an association between *E. coli* positive for *papC* with an increased uterine necrosis. Additionally, the degree of necrosis was positively associated with the length of hospital stay, with each increase in the degree of necrosis representing approximately two more days of hospitalization. Taken together, these findings reinforce the hypothesis that *papC* is strongly associated with pyometra caused by *E. coli*. In fact, a previous study showed that this virulence factor is directly associated with the adhesion and colonization of the canine endometrium and seems to facilitate the migration of bacteria present in the intestinal tract to the canine uterus, causing infection [49]. Our findings also support the idea that higher tissue necrosis, associated with the presence of *papC*, can directly impact disease severity, increasing the hospitalization time required. This is the first study to report an association between bacterial virulence factors and histopathological changes, as well as clinical outcomes.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.theriogenology.2023.10.007.

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Review

Canine Pyometra: A Short Review of Current Advances

Rafael Gariglio Clark Xavier, Clarissa Helena Santana, Yasmin Gonçalves de Castro, Thayanne Gabryelle Viana de Souza, Victor Santos do Amarante, Renato Lima Santos and Rodrigo Otávio Silveira Silva *

Veterinary School, Federal University of Minas Gerais, Antônio Carlos Avenue 6627, Belo Horizonte 31270-090, Brazil
* Correspondence: rodrigo.otaviosilva@gmail.com

Simple Summary: Pyometra is a common reproductive disease in dogs that often begins with mild symptoms, but if not promptly treated, it can turn into a threat to life. Despite being frequent, the disease is still not fully understood. In the last few years, studies have contributed to a better comprehension of this disease, raising new hypotheses regarding the epidemiology, bacteria involved, the pre-existing uterine lesions that might be associated, and even a possible influence of one's diet. In light of this, this work aimed to review the current understanding of canine pyometra, with particular emphasis on the recent research findings.

Abstract: Pyometra, characterized by the accumulation of purulent exudate in the uterus, is the most prevalent reproductive disease in canines. While the disease often begins with mild local symptoms, it can escalate into peritonitis, sepsis, and multi-organ dysfunction, thereby posing a significant threat to life. Despite the high incidence and recognized significance of canine pyometra, gaps persist in our understanding of its epidemiology, etiology, and pathogenesis. Recent studies have, however, broadened our comprehension of this disease, shedding light on potential new infection sources, etiologies, and the application of clinical predictive biomarkers and new therapeutic protocols. This study aimed to review the current understanding of canine pyometra, with particular emphasis on the latest research concerning its etiology and epidemiology. Furthermore, it addressed key research questions and proposed directions for future investigations into various facets of canine pyometra.

Keywords: reproductive; *Escherichia coli*; uterine

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1. Introduction

Pyometra is characterized by the accumulation of purulent exudate in the uterine lumen and is the most prevalent reproductive disease in canines [1]. It typically develops during the luteal phase, with *Escherichia coli* being the most frequently isolated bacteria [2–4]. Other commonly reported microorganisms include *Staphylococcus pseudintermedius* and *Streptococcus canis*. Recent studies, however, have suggested the potential involvement of less common pathogens, including *Brucella abortus*, *Corynebacterium* spp., and possibly *Porphyromonas* spp. [5,6].

Canine pyometra typically begins with subtle clinical signs such as polydipsia, polyuria, and vaginal discharge. Without timely treatment, it can progress to peritonitis, sepsis, and the dysfunction of multiple organs [7,8]. Consequently, it is regarded as a life-threatening infection [9–11].

Despite the prevalent occurrence and recognized significance of canine pyometra, our understanding of its epidemiology, etiology, and pathogenesis remains incomplete. Recent studies have broadened our knowledge of this disease, identifying potential new infection sources, causes, and biomarkers that could aid in predicting its prognosis and severity. Consequently, this review aimed to consolidate the current knowledge on canine pyometra, with particular emphasis on the latest research concerning its etiology and epidemiology.

2. Epidemiology and Risk Factors

Pyometra, a bacterial infection in the uterus, is the most prevalent reproductive disease in dogs, impacting up to 25% of non-castrated females [1]. This disease is characterized by a bacterial infection in the uterus that results in local and systemic clinical signs [10,12,13]. Although pyometra can occur in dogs ranging from 3 months to 20 years old, it predominantly affects middle-aged to older dogs (Figure 1), with a median diagnosis age of nine years [14–16]. The higher incidence of pyometra in middle-aged to older dogs is thought to be associated with repeated estrous cycles. During diestrus, progesterone enhances the secretory activity of the endometrial glands, promotes endometrial proliferation, diminishes myometrium contractility, and induces cervix closure [17]. Additionally, diestrus also reduces local leukocyte responses and uterine resistance to bacterial infection [1,18]. These effects, which accumulate after repeated estrous cycles, escalate the risk of pyometra with each cycle [14,17,19].

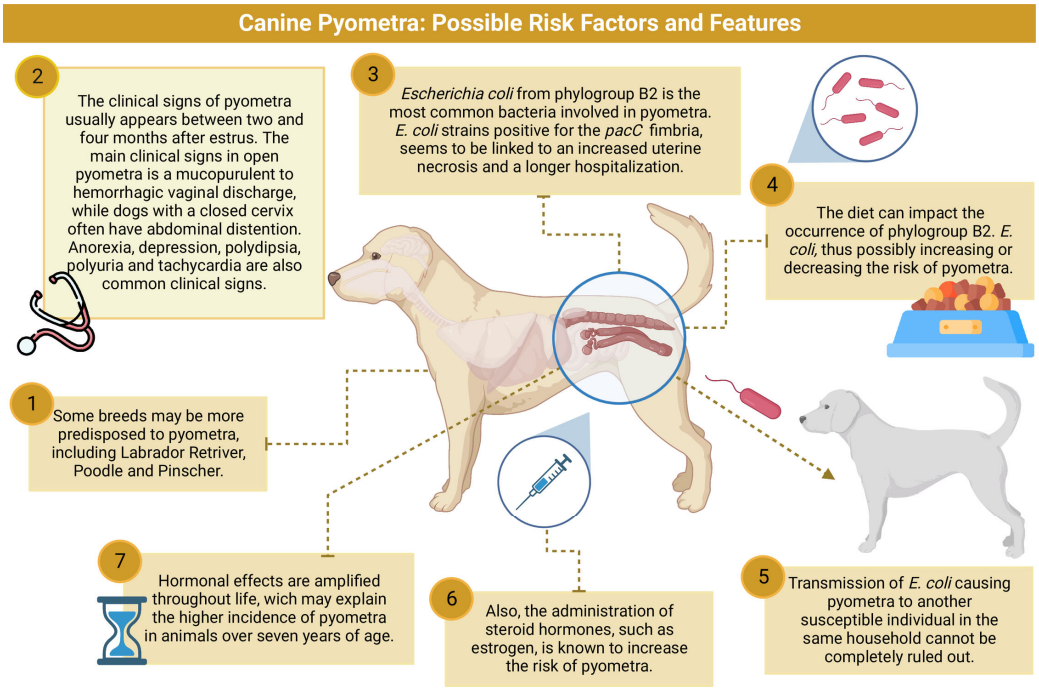

Figure 1. Infographic summarizing the possible risk factors and features of canine pyometra. 1—Some breeds may be more predisposed to pyometra [13,16,20]. 2—Although pyometra is primarily caused by microorganisms from the gastrointestinal tract, recent studies have suggested that other sources, including those that are hematogenous, are potential contributors [5,6]. 3—The most common bacteria involved in pyometra is phylogroup B2 *E. coli*, which ascend from the rectum microbiota to the uterus [21–23]. 4—Diet seems to impact the frequency of phylogroup B2 *E. coli* [21]. 5—Transmission of *E. coli*-causing-pyometra to another susceptible individual in the same household was recently reported [22]. 6—Administration of steroid hormones increases the risk of pyometra [23–26]. 7—A higher occurrence of pyometra is seen in animals around seven years of age, but the disease has been described in animals ranging in age from three months to 20 years [14–16,23]. Created using BioRender® (https://www.biorender.com/).

Some studies suggest that certain breeds may be more susceptible to pyometra (Table 1) [13,14,27,28]. However, the prevalence of pyometra appears to fluctuate according to studies conducted across various countries, and the hypothesis of breed predisposition remains speculative. Recent research involving Golden Retrievers has identified a potential correlation between pyometra and specific changes in the ABCC4 gene located on chromosome 22 [20,23]. This discovery introduces, for the first time, a potential explanation for the increased incidence of pyometra in a particular breed. Despite this finding, there remains

no definitive evidence of breed predisposition to pyometra, and the reasons for its higher prevalence in some breeds largely remain a mystery.

Table 1. Reported frequency of dog breeds affected by pyometra.

Breeds	Frequency (%)
Labrador Retriever	8–38
Poodle	10–33
Mixed-breed	27–30
Yorkshire Terrier	6–13
Pinscher	8–11
Golden Retriever	1–8
Rottweiler	1–8
Chow Chow	1–2
Others [1]	<1

[1] Other breeds include the American Pit Bull Terrier, Border Collie, German Shepherd, Lhasa Apso, Maltese, Pekingese, and Shih Tzu. Data from references [16,23,29–31].

The administration of drugs used for reproductive control, such as progestogens or estrogen compounds, is a recognized predisposing factor for canine pyometra [24–26,32]. These drugs, which suppress the sexual receptivity phase in female dogs, have been linked to an increased risk of pyometra and other conditions, including fetal maceration, endometrial and mammary tumors, and insulin resistance [26,33,34]. Hormonal effects, which intensify over time, may account for the higher incidence of pyometra in animals over seven years of age [15,23].

3. Etiopathogenesis

Despite the high incidence of canine pyometra, its pathogenesis remains inadequately understood. It is evident, however, that this pathogenesis is multifactorial, involving bacterial infection, hormonal changes (or a favorable endocrine environment), genetic predisposition, and pre-existing uterine lesions [35]. During the luteal phase of the estrous cycle (diestrus), progesterone stimulates the proliferation and secretion of endometrial glands. Moreover, progesterone inhibits myometrial contraction and weakens the uterine immune response, thereby promoting bacterial colonization [10,13,36]. Early studies on the pathogenesis of canine pyometra established a connection between hormonal stimulation and the occurrence of pyometra [32]. At that time, cystic endometrial hyperplasia was considered a predisposing endometrial lesion leading to pyometra under experimental conditions [32]. However, it was later discovered that, in addition to cystic endometrial hyperplasia, bitches in diestrus often develop another type of proliferative change in the endometrium. This change is characterized by endometrial hyperplasia with glandular cystic changes and decidual changes affecting the superficial endometrial epithelium, termed "pseudoplacentational endometrial hyperplasia" (Figure 2A,B) [37]. A recent study showed that in naturally occurring canine pyometra, pseudoplacentational endometrial hyperplasia is significantly associated with pyometra, whereas cystic endometrial hyperplasia is not [38]. Notably, despite this significant association, a cause-and-effect relationship between pseudoplacentational endometrial hyperplasia and pyometra has yet to be established [23,38]. These recent findings [38] suggest that the traditional terminology of the "cystic endometrial hyperplasia-pyometra complex" is outdated [35]. However, this should not be misinterpreted as diminishing the importance of endometrial hyperplastic changes in the pathogenesis of canine pyometra.

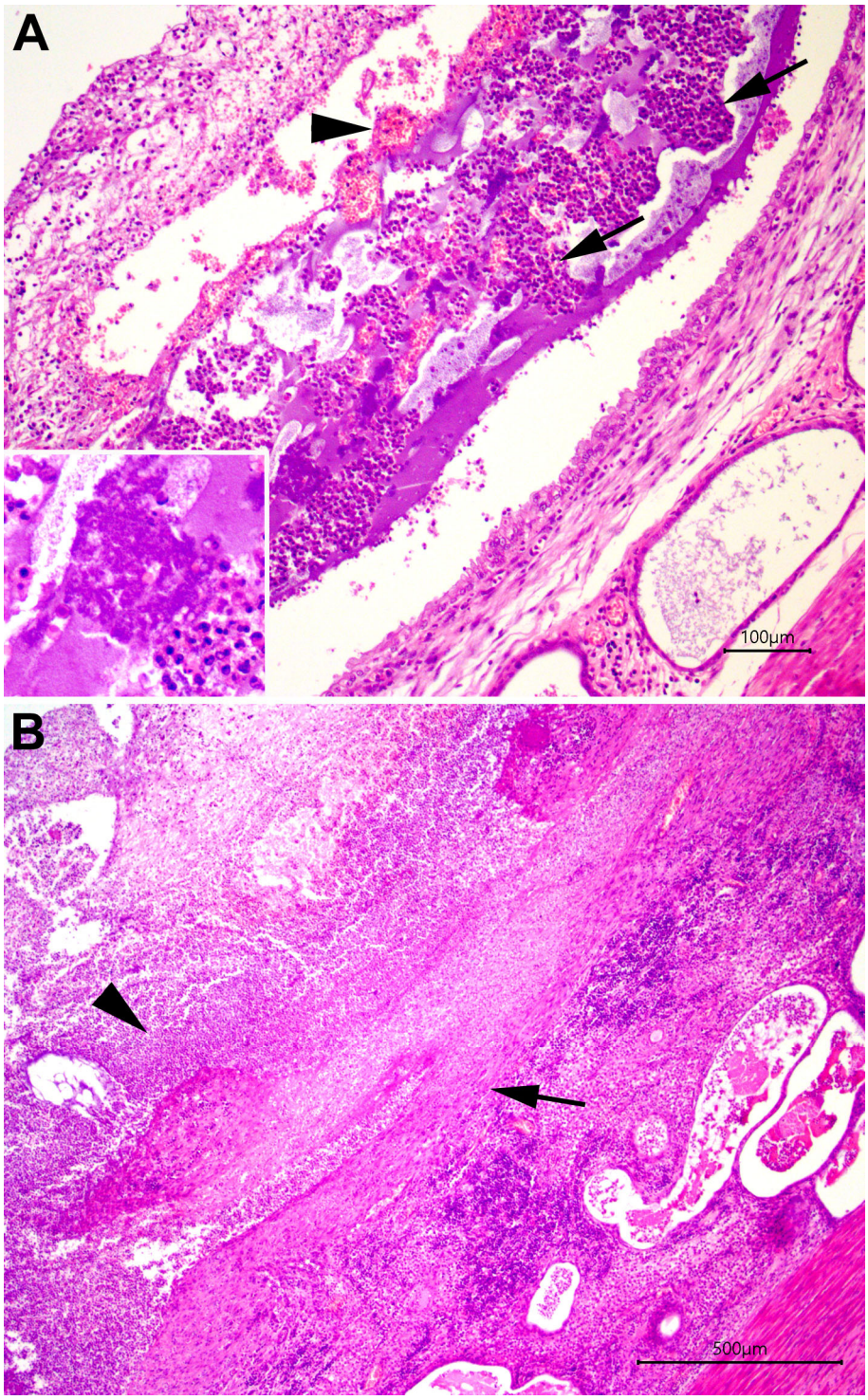

Figure 2. Uterus from a female dog with pyometra. (**A**) Endometrium with diffuse severe neutrophilic inflammatory infiltrates (arrows); hemorrhage (arrowhead), fibrin, and intraluminal bacterial aggregates (inset); and a columnar and vacuolated endometrial superficial epithelium (decidual reaction) and ectasia of endometrial glands in a case of pseudo-placentacional endometrial hyperplasia. HE; bar = 100 µm. (**B**) Endometrium with necrosis and superficial epithelial loss (arrow), with a diffuse severe neutrophilic inflammatory (arrowhead) infiltrate and mild hemorrhage. Endometrium with diffuse severe interstitial lymphoplasmacytic inflammation and marked glandular ectasia. HE; bar = 500 µm.

A broad spectrum of bacteria can contribute to pyometra in dogs [6,39]. *E. coli* is among the most prevalent microorganisms, implicated in up to 90% of canine pyometra cases (Table 2). This Gram-negative facultative anaerobic bacterium is also the primary pathogen in uterine infections across various species, including humans [4,40,41]. As *E. coli* is a component of the gut microbiota, it has been postulated that this microorganism can ascend from the rectum to the uterus, thereby causing this disease. This theory has been substantiated by studies demonstrating that the *E. coli* strains responsible for pyometra are often indistinguishable from those colonizing the gastrointestinal tract of the same dog [9,22,42]. Intriguingly, most dogs with pyometra are gut-colonized specifically by *E. coli* from phylogroup B2 [21], the same phylogroup frequently isolated from the uterine contents of affected animals [21,43,44]. Conversely, healthy dogs are more commonly gut-colonized by other phylogroups, including B1 [21,43,45]. This observation has led to the hypothesis that colonization by certain *E. coli* strains may elevate the risk of pyometra. In this context, a recent study demonstrated that diet can influence the colonization rate by *E. coli* from phylogroup B2 in the gut, suggesting that certain diets may indirectly heighten the risk of pyometra. If this hypothesis is further validated, strategies for altering or modulating the microbiota could provide an additional means of preventing or reducing the risk of pyometra [21].

Table 2. Most common bacterial species isolated from the uterus of female dogs with pyometra.

Organism	Frequency (%)
Escherichia coli	28–90
Staphylococcus sp.	2–42
Klebsiella pneumoniae	2–33
Streptococcus sp.	4–25
Proteus mirabilis	1–17
Pseudomonas aeruginosa	1–16
Enterobacter sp.	1–11
Enterococcus sp.	<1–3
No growth	10–26

Data from references [16,23,29–31].

In addition to phylogroup studies, researchers have examined the presence of virulence factors in *E. coli* isolated from canine pyometra. Some suggest that the possession of a specific combination of virulence genes may determine the severity of pyometra in female dogs [44,46]. Among these virulence factors, the gene encoding type P fimbriae (*papC*) has recently attracted considerable attention. Firstly, the prevalence of this gene is often higher in *E. coli* isolates from dogs with pyometra (ranging between 36.5 and 44.1%) compared to strains from the gut of healthy dogs (ranging between 18.2 and 29.2%) [21,47]. Secondly, experimental studies have shown that this fimbria plays a crucial role in the adhesion and colonization of *E. coli* in the canine endometrium [48]. A recent study also revealed a higher degree of uterine necrosis in dogs with pyometra caused by *E. coli papC*-positive strains. Interestingly, the degree of necrosis was positively correlated with the duration of hospitalization, suggesting a potential link between this fimbria and disease severity [16]. Another study proposed that uterine *E. coli* infection could alter the expression of sex hormone receptors in the uterus of female dogs, thereby enhancing the hormonal factors that promote bacterial growth [49]. Collectively, these studies strongly suggest that certain *E. coli* strains, possessing specific virulence traits, may be more likely to cause canine pyometra by facilitating tissue colonization and even modifying the uterine environment so that infection is favored. Further research is required to better understand the influence of the gut microbiota and diet on colonization by pyometra-causing *E. coli*.

In addition to *E. coli*, other members of the Enterobacteriaceae family, such as *Klebsiella pneumoniae* and *Proteus mirabilis*, are frequently implicated in pyometra. Bacteria from the *Streptococcus*, *Staphylococcus*, and *Enterococcus* genera are also noteworthy (Table 2). Studies have demonstrated that, similar to *E. coli*, *K. pneumoniae*, *S. pseudintermedius*, *S. canis*, and *E. faecalis* strains isolated from dogs with pyometra differ from most commensal strains. They express virulence factors such as adhesins, toxins, iron acquisition mechanisms, and mechanisms for evading the host immune system. These factors facilitate colonization and sustain the infection in the canine uterus [47,50–53].

Studies have intriguingly reported that no microorganisms are isolated in up to 25% of pyometra cases [8,21,54]. Several hypotheses have been proposed to explain this phenomenon, including the host immune system's elimination of the pathogen, the use of antimicrobials during the preoperative period, the low sensitivity of culture methods, and the existence of microorganisms that do not grow in the standard culture media used for routine diagnosis [54]. This last hypothesis has been reinforced by studies that have identified the presence of some uncommon microorganisms causing pyometra, such as *Mycoplasma* spp., *Nocardia* spp., *Corynebacterium* spp., *Moraxella* spp., *Clostridium perfringens*, *Porphyromonas* spp., and *Brucella abortus* [5,6]. While the infection in most cases likely ascends from the gastrointestinal tract, the detection of certain specific bacteria, including *Brucella abortus*, suggests that other infection routes, such as hematogenous pathways, are also possible [9,42,55]. Notably, *Porphyromonas* sp. has recently been confirmed as a cause of pyometra, leading to the hypothesis that bacteria typically found in the oral cavity can cause pyometra. Interestingly, *Porphyromonas* sp. is a well-established cause of reproductive diseases in humans as well as endocarditis, lung, liver, and kidney infections, which can spread through the bloodstream (hematogenously) [56–60].

4. Clinical Presentation

Pyometra typically manifests with local and systemic clinical signs (Figure 3), generally appearing between two and four-months post-estrus [9,10,61]. The most prevalent clinical symptom in dogs with open pyometra is the presence of vaginal discharge that ranges from mucopurulent to hemorrhagic in nature (Figure 4) [17]. Conversely, dogs with a closed cervix often exhibit abdominal distention owing to the lack of uterine content drainage (Figure 5) [7].

Clinical findings in pyometra cases can vary, but they commonly include inappetence/anorexia, depression/lethargy, polydipsia, polyuria, tachycardia, and tachypnea [8,11,17]. Pyometra is a life-threatening condition because of the potential for complications such as uterine rupture, nephropathy, peritonitis, endotoxemia, and, particularly, sepsis [13,38,62].

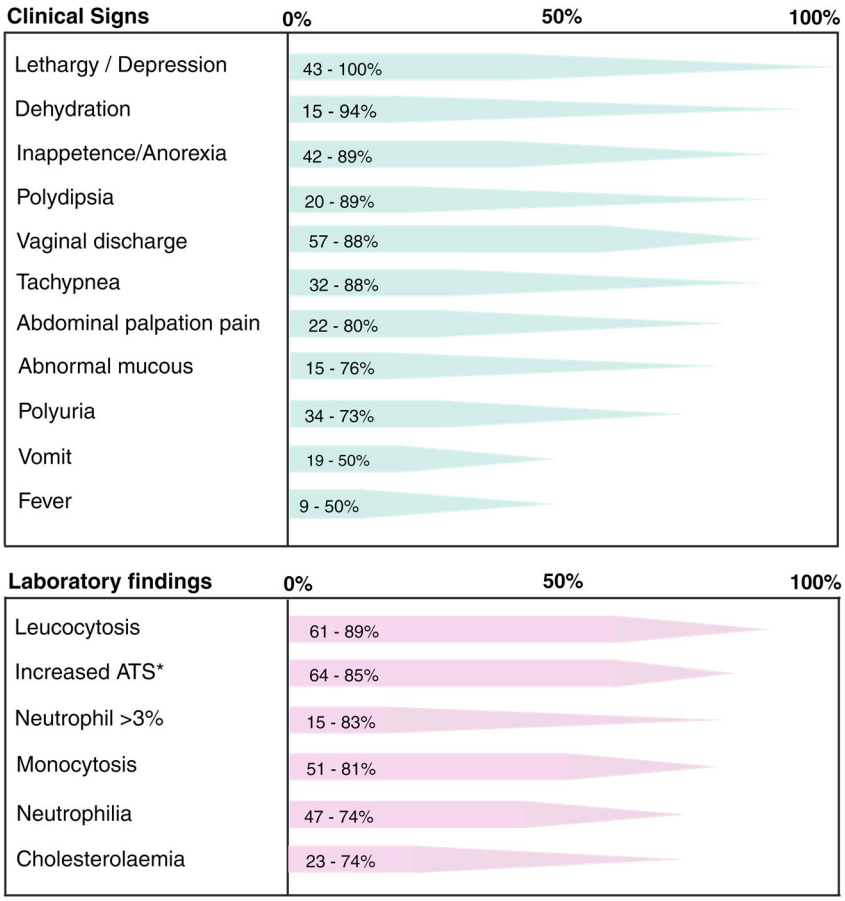

Figure 3. Main clinical signs and laboratory findings in female dogs with pyometra, according to previous reports [7,11,16,23,63–66]. * AST—Aspartate aminotransferase.

Figure 4. Purulent vaginal discharge in a bitch with open cervix pyometra.

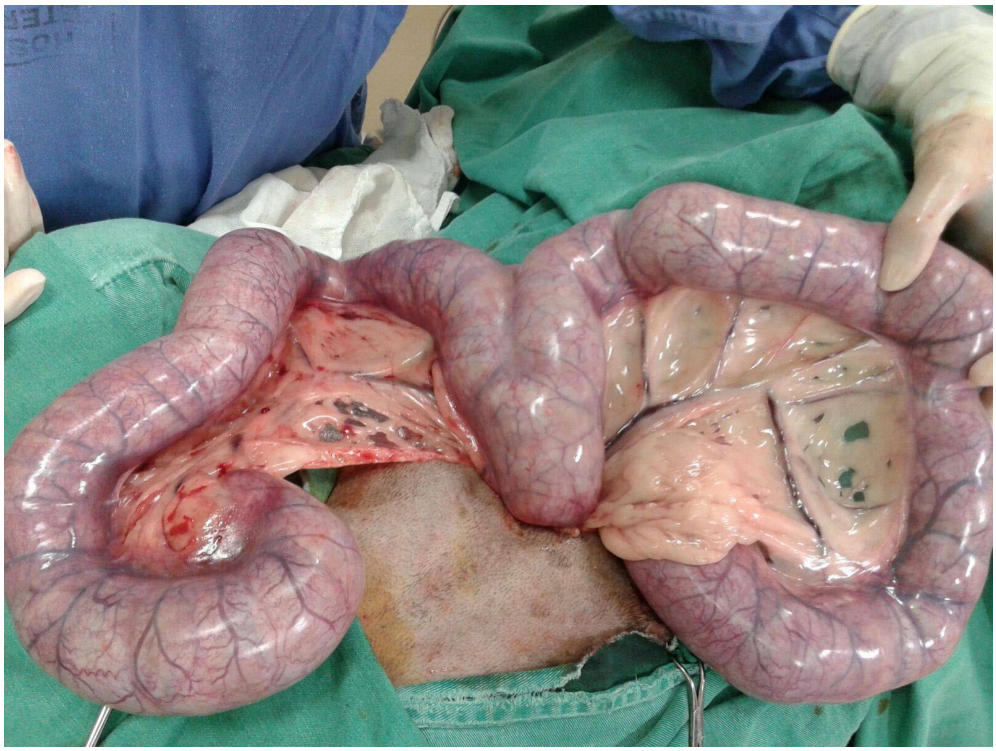

Figure 5. Intraoperative image of an enlarged, pus-filled uterus in a bitch (mixed-breed dog) with pyometra.

Notably, fever and hypothermia have been identified as factors increasing the risk of peritonitis development. Concurrently, moderate to severe general depression and pale mucous membranes are linked to extended hospitalization periods [11]. Furthermore, animals with closed pyometra exhibit a more severe condition and an elevated risk of sepsis [7,67].

5. Diagnosis

The clinical diagnosis of this disease is often made for cases of open pyometra. However, in the absence of vaginal discharge, diagnosis can be significantly more challenging owing to the variability of other clinical signs [8]. Typically, diagnosis relies on patient history, clinical signs, and imaging tests such as abdominal radiography and ultrasound. Additional tests, including blood counts, leukograms, and liver function evaluations, can also provide valuable information (Figure 3) [11,13,44]. Leukocytosis and anemia, along with signs of azotemia, are frequently observed in affected animals. This is because renal dysfunction can result from endotoxemia, glomerular dysfunction, renal tubular damage, and a decreased response to the antidiuretic hormone [8].

Ultrasonography has proven beneficial in identifying intrauterine fluid, even when the uterine diameter falls within the normal range (Figure 6). Additionally, it offers the advantage of revealing further pathological alterations in the tissue and ovaries, such as ovarian cysts or cystic endometrial hyperplasia [8,68].

While not commonly requested, additional complementary examinations may prove beneficial. These include histopathological analyses of the uterus following ovariohysterectomy and a microbiological culture of uterine content. These tests can confirm a diagnosis of pyometra, identify the bacteria associated with the infection, and facilitate antimicrobial susceptibility testing of the isolate [23].

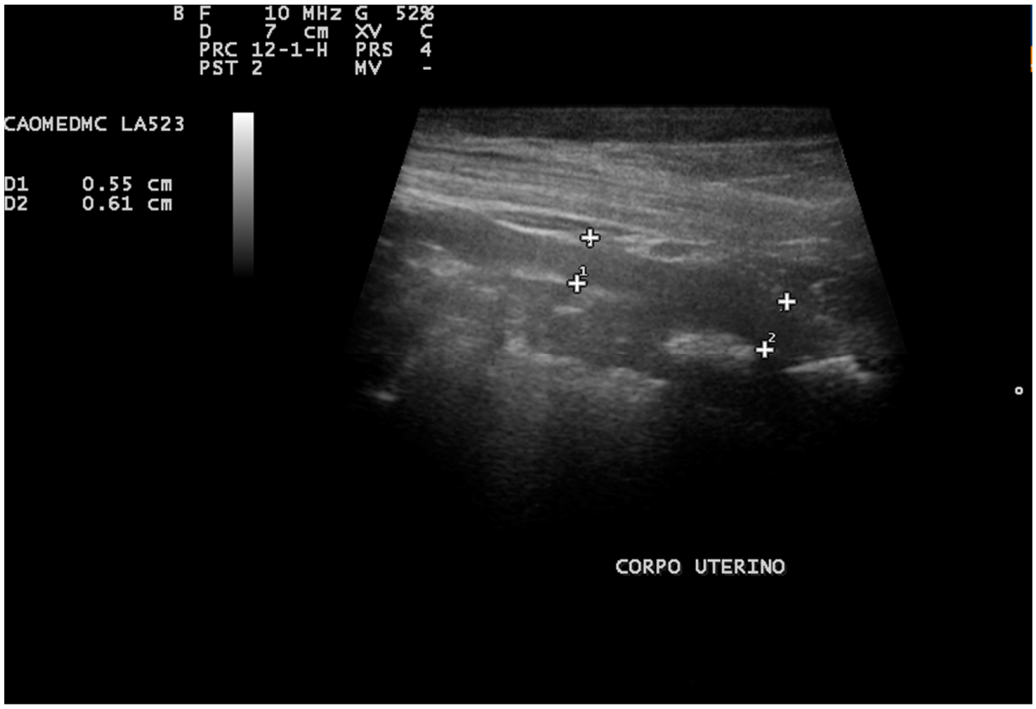

Figure 6. Abdominal ultrasound image of the uterus of a Pinscher. An enlarged left uterine horn measuring approximately 4.26 cm in diameter in the transverse plane is noted (cursors), with hypoechogenic content related to pyometra.

6. Treatment

Pyometra is a medical emergency requiring prompt attention, and ovariohysterectomy (OHE) remains the preferred treatment option. Typically, a patient's overall clinical condition reverts to normal within two weeks once the infection source is eliminated [7,9]. However, the procedure's primary drawback is permanent sterility, which is particularly significant if the owner has a breeding interest in the animal [10]. Complications associated with OHE include hemorrhage, accidental ureteral ligation, estrogen-responsive urinary incontinence, ovarian remnant syndrome, and stump pyometra [69,70]. Stump pyometra may develop post-OHE if a section of the uterine horns or body remains and the animal exhibits elevated progesterone levels and/or an ovarian remnant [69–71]. The clinical manifestation, diagnosis, and treatment of stump pyometra are similar to those for pyometra, except for the history of a prior OHE [23].

While antibiotic therapy is frequently incorporated into the standard treatment protocol for pyometra, some researchers propose that perioperative antimicrobials should be reserved for animals exhibiting moderate to severe depression, thereby minimizing unnecessary antimicrobial usage [72,73]. In such instances, the initial selection of an antimicrobial should be effective against *E. coli*, the most prevalent bacteria implicated, and, ideally, adjusted based on culture and antibiogram results to a personalized narrow-spectrum alternative for each patient, thereby mitigating the risk of selecting multidrug-resistant bacteria [46,74]. However, it is worth noting that the majority of veterinarians seldom, if ever, request these culture tests [75].

Fluoroquinolones, such as enrofloxacin and amoxicillin/clavulanate, are the primary and secondary recommendations for pyometra treatment according to the Antibiotic Use Guidelines for Companion Animal Practice (Table 3) [76]. Conversely, the Finnish and Swedish guidelines propose sulfadoxine-trimethoprim and ampicillin as the preferred choices, respectively [72,77]. Research from various countries indicates that most antimicrobials, including those recommended by these guidelines, are largely effective against isolates from canine pyometra. Other effective compounds include cephalothin, streptomycin, and gentamicin [9,15,78–80]. A recent retrospective review corroborated these

findings by demonstrating that ampicillin or amoxicillin are effective antimicrobials for cases requiring antibiotic treatment, particularly in dogs exhibiting moderate to severe general demeanor depression [73].

Table 3. Antimicrobials recommended for the treatment of bitches with pyometra, according to published guidelines and references [72,76,77].

Drugs	Dosage	Reference
Sulfadoxine-trimethoprim	15 mg/kg/q 12 h	[72]
Ampicillin	10–20 mg/kg/q 6–8 h	[77]
Enrofloxacin	2.5–5.0 mg/kg/q 12 h	[76]
Amoxicillin/clavulanate	10–20 mg/kg/q 12 h	

Pharmacological treatment has been exclusively utilized in certain scenarios, such as with young breeders or when anesthesia and surgery are currently not feasible [7,81,82]. The goal of the pharmacological management of pyometra is to actively expel purulent contents from the uterus and inhibit bacterial growth, thereby promoting uterine healing. Consequently, these protocols typically involve the simultaneous administration of steroids, antiprogestatives, and antimicrobials (Table 4). Aglepristone, a progesterone receptor blocker, and cloprostenol, a synthetic prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) analog, are commonly used in this regard [10,83,84].

Table 4. Examples of protocols used for pharmaceutical treatment of open cervical pyometra affecting bitches.

Drugs	Dosage	Frequency	Reference
Aglepristone	10 mg/kg q 24 h	Three doses. Days 1, 2, and 7 or 2, 7 and 14 or 1, 2 and 7 Four doses. Days 1, 3, 6 and 9	[84–86]
Aglepristone Cloprostenol	10 mg/kg q 24 h 1 µg/kg SC q 24 h	Days 1, 3, 8 and 15 Days 3 and 8	[87]

In addition, antimicrobial therapy (preferably based on sensitivity tests) and supportive treatment are essential. It is important to note that these protocols are not recommended for dogs exhibiting certain clinical signs, such as fever, hypothermia, liver and/or kidney failure, or suspected peritonitis. Bitches subjected to nonsurgical treatment need to be closely monitored considering the risk of drug side effects and rapid general health deterioration, the latter of which is mostly linked to sepsis and endotoxemia. Also, owners should be aware that recurrence is possible. Interestingly, it was once believed that, following pharmacological treatment, endometrial lesions in canine pyometra would impair a dog's ability to conceive or sustain pregnancy, but recent studies show that the pregnancy rate and litter size do not decrease [1,10,82,88,89].

7. Predictive Markers

Research has attempted to link prognosis with the identification of certain biomarkers [90]. Factors such as leukopenia, inappetence, azotemia, reduced packed cell volume, and dehydration have been correlated with extended hospitalization following OHE [23,64,90]. Additionally, leukopenia has been connected with the incidence of peritonitis [11,23].

C-reactive protein (CRP) is arguably the most extensively researched biomarker in dogs with pyometra. Current knowledge suggests that CRP levels decrease gradually following OHE, with sustained or increased concentrations potentially indicating complications [23,90,91]. Similarly, serum amyloid A, the hormone procalcitonin, and

cell-free DNA exhibit the same pattern [23,90–92]. CRP levels are also elevated in dogs with pyometra and sepsis compared to those with mucometra [93]. Consequently, some researchers have proposed that CRP could serve as a marker for severe cases or be used to distinguish pyometra from mucometra [17,94,95]. The level of CRP has been directly linked to the length of the postoperative period [96], suggesting its potential as a valuable prognostic tool. Other studies have proposed that serum amyloid A and cell-free DNA could be used for sepsis screening, while interleukin-6 and high-mobility group Box 1 might be useful for the therapeutic monitoring of sepsis [67,90,96]. Regrettably, these biomarkers are not yet routinely used in most veterinary hospitals. Conversely, some parameters commonly included in routine testing, such as serum creatinine and urinary gamma-glutamyl transpeptidase, have not proven clinically useful in determining the severity of pyometra or renal injury in affected dogs [97].

8. Prevention

Elective OHE ("spaying") serves as the primary method for pyometra prevention. However, sterilization can lead to adverse side effects, including surgical and anesthetic complications, a heightened occurrence of certain musculoskeletal and endocrinological disorders, obesity, and urinary incontinence in female dogs [98]. It is crucial to meticulously evaluate the advantages and disadvantages of such a procedure in each case, considering the breed of the animal [99–101].

The potential for pathogenic *E. coli* to ascend from the intestinal tract to the uterus has been documented [21,23]. This finding suggests that future research could explore how various diets influence intestinal colonization by bacteria that cause pyometra, potentially leading to preventative measures for this condition in dogs. A recent case study reported the transmission of pyometra between two Chow Chow dogs. Although the mechanisms are not fully understood, it is suggested that isolating healthy cohabiting animals from dogs with purulent vaginal discharge (indicative of open-cervix pyometra) may prevent disease transmission [22].

9. Future Perspectives

Canine pyometra, a potentially lethal and commonly occurring reproductive disease in female dogs, is known to be influenced by preexisting uterine lesions and hormonal and bacterial factors. However, its pathogenesis remains largely unknown. Our understanding of the etiological factors involved in *E. coli*-induced pyometra as well as the role of other pathogens is continually evolving. Future research may elucidate the influence of diet and intestinal microbiota on the risk of pyometra, potentially aiding in the development of more effective prevention protocols for this enduringly prevalent disease. Although challenging, it is necessary to determine whether certain breeds are at a higher risk of developing pyometra. Understanding the mechanisms underlying this susceptibility can help in developing novel strategies for preventing and reducing the incidence of pyometra.

Recent studies have also described the involvement of less common pathogens in pyometra, raising the hypothesis that other infection routes, including hematogenous routes, may be more common than previously anticipated. In this context, the hypothesis that microorganisms from oral microbiota cause pyometra should be further explored.

10. Conclusions

Despite the significance of canine pyometra, our understanding of its epidemiology and etiopathogenesis remains limited. Pyometra is a multifactorial disease that commonly occurs during diestrus. Some established risk factors include age and the use of drugs for reproductive control. It is also likely that some breeds are predisposed to infection; however, this hypothesis has not yet been proven. Bitches with cystic endometrial hyperplasia or pseudo-placentational endometrial hyperplasia also seem to be at higher risk of developing infections. Ovariohysterectomy (OHE) remains the preferred treatment for

canine pyometra; however, pharmacological treatment, commonly with anglepristone, is also possible in some specific cases.

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CERTIFICADO DE MENÇÃO HONROSA

A Comissão Organizadora do Colóquio tem a grande satisfação de conferir este certificado de Menção Honrosa pelo brilhante resumo científico, apresentado no IX Colóquio Técnico Científico de Saúde Única, Ciências Agrárias e Meio Ambiente:

PRIMEIRO RELATO DE TRANSMISSÃO DE PIOMETRA ENTRE DUAS CADELAS COABITANTES

De autoria de

Isabela Pádua Zanon, Clara Alcântara Lara de Mesquita, Giulia Said Oliveira, Isadora Maria Soares de Melo, Rafael Gariglio Clark Xavier, Rodrigo Otávio Silveira Silva

PRHISCYLLA SADANÃ PIRES

Gestora acadêmica de Ciências Agrárias e Meio Ambiente do Ecossistema Ânima

GABRIEL ALMEIDA DUTRA

Coordenador Regional MG/GO de Ciências Agrárias e Meio Ambiente do Ecossistema Ânima

CERTIFICADO DE RELEVÂNCIA ACADÊMICA

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PRHISCYLLA SADANÃ PIRES

Gestora acadêmica de Ciências Agrárias e Meio Ambiente do Ecossistema Ânima

GABRIEL ALMEIDA DUTRA

Coordenador Regional MG/GO de Ciências Agrárias e Meio Ambiente do Ecossistema Ânima