



**Politécnico
de Viseu**

Escola Superior
Agrária de Viseu

Optimizing Antibiotic Use in the Treatment of Pyometra: Preventing Antimicrobial Resistance

Daniela Andrade Pancrácio

Project-Final Version

Master's Degree in Veterinary Nursing – Companion Animals

Viseu, 2026



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Projeto- Versão Final

Mestrado em Enfermagem Veterinária de Animais de Companhia

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ACKNOWLEDGMENTS

First, I would like to express my sincere gratitude to the entire FMV-ULHT hospital team for their constant support, availability in case selection, and truly collaborative spirit, which made this process lighter and more enriching.

To Dr. João Martins, my external supervisor and head surgeon, I owe a very special thank you for his tireless guidance, the trust he placed in me, and his unfailing encouragement. Thank you for believing in my work and for pushing me to go further every time.

To my Junior and Interns surgery team, who truly feel like family, thank you for your support, friendship, and companionship. To the senior surgeons and anaesthetists, I am deeply grateful for your generosity in sharing your knowledge and for your help at every stage of this journey.

To the entire nursing team, and especially my fellow surgery nurses, thank you for your support, patience, and good humour, which make daily work so much lighter.

To my family, especially my parents, thank you for standing by me through all the ups and downs of my decision to continue my academic path while working in an already demanding profession.

To my younger sister, Inês, who is growing into an incredible person, always present and ready to teach me a few tech tricks, thank you for being exactly who you are.

To Pablo, thank you for believing in me even when I doubt myself, for your constant motivation and support, and for being my peace in the middle of every storm.

To my friends, both long-standing and more recent, thank you for understanding my absences and for celebrating my achievements. A special thank you to my friends in Viseu for welcoming me with open arms whenever I needed to return.

To all the professors at ESAV, I extend my sincere appreciation for your support, kindness, and approachability, which create a truly family-like environment.

Finally, to my supervisor, Professor Maria Aires Pereira, who, from the very beginning of this master's, awakened in me a passion for research, thank you for your authenticity and attentiveness. Your guidance was essential for this work to become a reality.

RESUMO

A piómetra em cadelas e gatas é uma infecção uterina frequente e potencialmente fatal, sendo um dos principais motivos de utilização de antibióticos em animais de companhia. Este estudo teve como objetivo caracterizar a microbiota vaginal e uterina de fêmeas com piómetra e avaliar se as culturas vaginais podem orientar de forma fiável a antibioterapia empírica. Realizou-se um estudo retrospectivo, observacional e transversal num hospital universitário, incluindo 39 fêmeas inteiras (27 cadelas, 69,2%; 12 gatas, 30,8%), nas quais se obtiveram zaragatoas emparelhadas da vagina e do útero para cultura aeróbia, sequenciação do gene 16S rRNA e testes de suscetibilidade por difusão em disco. *Escherichia coli* foi o agente mais frequentemente isolado em ambas as espécies e locais de colheita, destacando-se nas amostras uterinas de cadelas (78,6%). Nos casos caninos com amostras emparelhadas (n = 34), verificou-se concordância entre os agentes vaginais e uterinos em 18 casos (52,9%), enquanto 16 (47,1%) apresentaram isolados discordantes; nas gatas (n = 11), a concordância foi de 63,6% (7/11) e a discordância de 36,4% (4/11). Os padrões de suscetibilidade antimicrobiana foram globalmente favoráveis, com a maioria dos isolados a manter suscetibilidade a cefovecina, amoxicilina-clavulanato, gentamicina e cefalosporinas, em linha com a literatura recente. Estes resultados sugerem que as culturas vaginais podem fornecer informação útil mas incompleta sobre a infecção uterina, reforçando a necessidade de colheitas uterinas sempre que possível e evidenciando oportunidades para otimizar a antibioterapia empírica e promover o uso responsável de antibióticos no tratamento da piómetra.

PALAVRAS-CHAVE: piómetra; microbiota vaginal; microbiota uterina; infecção; multirresistência; antibióticos

ABSTRACT

Pyometra in bitches and queens is a common and potentially life-threatening uterine infection and a major indication for antibiotic use in companion animals. This study aimed to characterize the vaginal and uterine microbiota of females with pyometra and to assess whether vaginal cultures can reliably guide empirical antimicrobial therapy. A retrospective, observational, cross-sectional study was conducted at a university veterinary hospital, including 39 intact females (27 dogs, 69.2%; 12 cats, 30.8%), from which paired vaginal and uterine swabs were collected for aerobic culture, 16S rRNA gene sequencing, and disk diffusion susceptibility testing. *Escherichia coli* was the most frequently isolated bacterial species across both species and sampling sites, particularly in uterine samples from bitches (78.6%). In canine cases with paired samples (n = 34), agreement between uterine and vaginal bacterial agents was observed in 18 cases (52.9%), whereas 16 cases (47.1%) showed discordant isolates; in queens (n = 11), uterine and vaginal isolates agreed in 7 cases (63.6%) and were discordant in 4 cases (36.4%). Antimicrobial susceptibility patterns were generally favourable, with most isolates remaining susceptible to cefovecin, amoxicillin–clavulanate, gentamicin and cephalosporins, in accordance with recent reports on small-animal pathogens. These findings indicate that vaginal cultures can provide useful but incomplete information about uterine infection, underscoring the importance of uterine sampling whenever feasible and highlighting opportunities to optimize empirical antimicrobial therapy and promote responsible antibiotic use in the management of pyometra.

KEYWORDS: pyometra; vaginal microbiota; uterine microbiota; infection; multidrug resistance; antibiotics

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GLOSSARY OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

AMR:	Antimicrobial resistance
AST:	Antimicrobial susceptibility testing
AVMA:	American Veterinary Medical Association
CDC:	Centers for Disease Control and Prevention
CEH:	Cystic endometrial hyperplasia
CLSI:	Clinical and Laboratory Standards Institute
COX-2:	Cyclooxygenase-2
EMA:	European Medicines Agency
ESBL:	Extended-spectrum β -lactamase
EUCAST:	European Committee on Antimicrobial Susceptibility Testing
FMV-ULHT:	Faculdade de Medicina Veterinária da Universidade Lusófona de Humanidades e Tecnologias
FSH:	Follicle-stimulating hormone
GnRH:	Gonadotropin-releasing hormone
LAB:	Lactic acid-producing bacteria
LH:	Luteinizing hormone
LPS:	Lipopolysaccharides
MA:	Megestrol acetate
MDR:	Multidrug-resistant
MIC:	Minimum inhibitory concentration
MPA:	Medroxyprogesterone acetate
MRSP:	Methicillin-resistant <i>Staphylococcus pseudintermedius</i>
OVH:	Ovariohysterectomy
PBP:	Penicillin-binding protein
PRG:	Proligestone
PU/PD:	Polyuria/polydipsia
R:	Resistant
I:	Intermediate
S:	Susceptible
SIRS:	Systemic inflammatory response syndrome
VCIA:	Veterinary critically important antimicrobials
VEGF:	Vascular endothelial growth factor
VHIA:	Veterinary highly important antimicrobials
VIA:	Veterinary important antimicrobials
WHO:	World Health Organization
WOH:	World Organisation for Animal Health

INTRODUCTION

Canine and feline pyometra represents one of the most common and life-threatening reproductive disorders in intact female companion animals, affecting approximately 20–25% of intact bitches by ten years of age (Egenvall et al., 2001; Hagman, 2022). It typically occurs during diestrus due to prolonged progesterone exposure, which induces endometrial proliferation, glandular secretion, reduced myometrial tone, and local immunosuppression (Hoffmann et al., 2004; Smith, 2006). These changes can lead to cystic endometrial hyperplasia (CEH), a frequent histopathological finding (De Bosschere et al., 2001).

Clinically, pyometra is characterized by the accumulation of purulent material within the uterine lumen and may initially present with non-specific signs such as polyuria, polydipsia, lethargy, anorexia, vomiting, and vaginal discharge; if left untreated, it can rapidly progress to sepsis, peritonitis, multi-organ failure, and death (Hagman, 2022, 2023).

Pyometra results from an ascending bacterial infection, usually originating from the host's own vaginal or intestinal flora (Sandholm et al., 1975). *Escherichia coli* is the most frequently isolated pathogen, often exhibiting virulence factors distinct from those found in commensal or enteropathogenic strains (Xavier et al., 2024). Other implicated bacteria include *Staphylococcus pseudintermedius*, *Streptococcus canis*, *Pseudomonas* spp., *Proteus* spp., and *Klebsiella* spp., in pure or mixed cultures (Huber et al., 2022; Hagman, 2023).

Ovariohysterectomy (OVH) remains the gold-standard treatment for pyometra, as it removes the infected uterus and ovaries, resolves the systemic inflammatory response and prevents recurrence (Hagman, 2023). However, the optimal use of perioperative and therapeutic antimicrobials in these patients is still controversial. Swedish national guidelines, for example, recommend omitting prophylactic antibiotics in systemically stable dogs undergoing OVH for pyometra, provided that strict aseptic technique is observed (Turkki et al., 2023). In contrast, many clinicians routinely administer broad-spectrum antibiotics pre-, intra- and postoperatively, often in the absence of culture and susceptibility data (Lavin & Maki, 2023). There is likewise no clear consensus regarding the most appropriate antimicrobial classes, dosing regimens, or treatment durations for these cases (Ylhäinen et al., 2023).

The emergence and spread of antimicrobial resistance (AMR) among companion-animal pathogens represent a growing concern in veterinary medicine. Increasing rates of resistance have been documented in Gram-negative and Gram-positive isolates recovered from canine pyometra, including multidrug-resistant (MDR) *E.coli* strains (Agostinho et al., 2014; Inoue et al., 2013; Rocha et al., 2022). Empirical and often prolonged antimicrobial use, especially when not guided by culture and antimicrobial susceptibility testing (AST), may further select for resistant bacteria and disrupt the normal microbial ecosystem of the reproductive tract (Groppetti et al., 2012)

Despite the high clinical and welfare impact of pyometra, relatively few studies have concurrently evaluated bacterial flora from both vagina and uterus, treatment practices, and resistance patterns using standardized methodologies. Further research is therefore needed to optimize therapeutic strategies, limit unnecessary antimicrobial exposure, and reduce the risk of resistance development in this common reproductive emergency (Weese et al., 2015, 2018).

1. LITERATURE REVIEW

1.1 Reproductive tract of female dogs and cats

The female reproductive tract of dogs (*Canis lupus familiaris*) and cats (*Felis catus*) shares the same basic anatomical organization, consisting of paired ovaries, oviducts, a bicornuate uterus, cervix, vagina, and vulva (figure 1). This arrangement is typical of polytocous domestic carnivores and is anatomically adapted to support multiple fetuses along elongated uterine horns (Johnston et al., 2001; England & Russo, 2006). Although the overall layout is similar in both species, there are important morphological particularities that have implications for reproduction, diagnostic imaging, and surgical approaches, as well as for the pathogenesis of uterine disease such as pyometra (Johnston et al., 2001; Hedlund, 2013).

The ovaries are paired, ovoid organs located in the dorsal abdomen, caudal to the respective kidneys and suspended within a peritoneal fold, the mesovarium. In the bitch, the ovaries are relatively large and are completely or partially enclosed by the ovarian bursa, a peritoneal pouch formed by the mesovarium and mesosalpinx that contains variable amounts of fat (Johnston et al., 2001). The suspensory ligament of the ovary anchors each ovary to the last rib region and must often be stretched or broken during ovariohysterectomy, representing a key surgical landmark (Hedlund, 2013).

In the queen, the ovaries are proportionally smaller, and the ovarian bursa is less extensive than in the bitch, though it still forms a protective pocket around the ovary and the proximal oviduct (England & Russo, 2006). Histologically, in both species, the ovary is composed of an outer cortex, containing follicles at different stages of development and corpora lutea, and an inner medulla rich in blood vessels, lymphatics, and nerves (Johnston et al., 2001).

The oviducts, or uterine tubes, are narrow, convoluted ducts that extend from the ovary to the uterine horn. Each tube is divided into the infundibulum, ampulla, and isthmus. The infundibulum, with its fimbriae, closely associates with the ovary and captures the oocyte at ovulation; the ampulla is the usual site of fertilization, whereas the isthmus connects to the uterine horn at the uterotubal junction (Verstegen et al., 2008). The oviductal mucosa is lined by ciliated and secretory cells that facilitate gamete and early embryo transport (Verstegen et al., 2008).

Both bitches and queens possess a bicornuate uterus characterized by a short uterine body and two long uterine horns, a configuration that accommodates multiple fetuses in each gestation (Johnston et al., 2001). In the bitch, the uterine horns are comparatively shorter and broader; in the queen, they are proportionally longer and slenderer, often extending cranially beyond the mid-abdomen. These differences influence the radiographic and ultrasonographic appearance of the uterus and can affect surgical access during OVH or caesarean section (England & Russo, 2006; Hedlund, 2013).

The uterine wall in both species is composed of three concentric layers: a serosal outer layer (perimetrium), a thick muscular middle layer (myometrium) and an inner mucosal layer (endometrium). The myometrium consists of interlacing smooth muscle bundles responsible for uterine contractions during parturition and for mechanical clearance of uterine contents. The endometrium is lined by simple columnar epithelium with tubular glands that undergo cyclical changes under the influence of ovarian steroids. During diestrus, prolonged progesterone stimulation leads to endometrial proliferation, glandular hypersecretion and reduced myometrial contractility, creating the conditions for cystic endometrial hyperplasia and, in susceptible animals, pyometra (De Bosschere et al., 2001; Hagman, 2022).

The cervix is a short, firm muscular canal that forms the caudal end of the uterus and opens into the cranial vagina. In the bitch, the cervix is relatively long and prominent, projecting into the vaginal lumen as a distinct cervical portion that can sometimes be palpated or visualized endoscopically (Johnston et al., 2001). In queens the cervix tends to be shorter and less protuberant, making endoscopic identification more challenging (England & Russo, 2006).

Structurally, the cervix contains dense connective tissue and smooth muscle arranged predominantly in circular layers, with a narrow central lumen that is normally tightly closed except in estrus and parturition. The mucosa is lined by columnar epithelium cranially and transitions to stratified squamous epithelium caudally, merging with the vaginal lining. Cervical glands secrete mucus that forms a physical and biochemical barrier to ascending bacteria for most of the cycle, whereas during estrus the cervix relaxes and partially opens to allow passage of spermatozoa, temporarily increasing susceptibility to ascending infection (Davidson et al., 2016; Nascimento et al., 2024).

The vagina is a distensible fibro-muscular tube extending from the cervix to the level of the urethral tubercle, where it continues caudally as the vestibule. In the bitch, the vagina is relatively long and spacious, with prominent longitudinal folds that facilitate expansion during coitus and parturition (Johnston et al., 2001). In the queen, the vagina is proportionally shorter and narrower, which is clinically relevant for vaginal cytology and endoscopic procedures (England & Russo, 2006).

The vaginal mucosa is lined by stratified squamous epithelium that undergoes marked, cycle-dependent changes in thickness and keratinization. During proestrus and estrus, the epithelium becomes thickened and increasingly keratinized, a feature routinely exploited in vaginal cytology to stage the estrous cycle (Concannon, 2011). The lamina propria contains rich vascular and lymphatic networks but lacks true vaginal glands; most vaginal secretions originate from cervical and vestibular glands (Johnston et al., 2001).

Caudal to the vaginal fornix lies the vestibule, a shared passage for the urinary and genital tracts. The external urethral orifice opens on the urethral tubercle at the junction between vagina and vestibule. The vestibule terminates at the vulvar lips and is lined by stratified squamous epithelium, with numerous minor vestibular glands that contribute to lubrication (England & Russo, 2006).

The anatomic configuration of the female reproductive tract in bitches and queens underpins both normal reproductive physiology and the development of disease. The long uterine horns and bicornuate uterus explain the high fecundity of these polytocous species, whereas the structure and function of the cervix and endometrium are central to the pathogenesis of pyometra. Progesterone-induced cervical closure and reduced myometrial activity impair uterine drainage, while endometrial gland proliferation and secretion provide an ideal medium for bacterial growth once pathogens ascend from the vagina (Hagman, 2022).



Figure 1- Anatomic diagram of both female canine (A) and feline (B)

The reproductive cycle in both species shares the same regulatory foundation, being controlled by the hypothalamic-pituitary-gonadal axis and characterized by cyclical fluctuations of gonadotropins and ovarian steroids (Johnston et al., 2001; Concannon, 2011). Both species exhibit distinct stages, including proestrus, estrus, diestrus, and anestrus, with external signs and behavioral modifications reflecting underlying endocrine activity. Follicular development, ovulation, and corpus luteum formation occur in both species, ensuring the possibility of conception during estrus (Verstegen et al., 2008).

Both species rely on the hypothalamic–pituitary–gonadal axis for reproductive control. Gonadotropin-releasing hormone (GnRH), secreted by the hypothalamus, stimulates the anterior pituitary to release follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH drives follicular growth and maturation within the ovaries, while LH triggers final follicular maturation and ovulation. Estrogen, produced by developing follicles, mediates behavioral estrus and prepares the reproductive tract for mating, whereas progesterone, secreted by the corpus luteum following ovulation, maintains diestrus and supports pregnancy if conception occurs (Johnston et al., 2001; Concannon, 2011).

Despite these shared mechanisms, the timing, frequency, and hormonal dynamics of the estrous cycle vary markedly between the two species, with important consequences for reproductive health and disease susceptibility (England & Russo, 2006; Verstegen et al., 2008).

The bitch is a spontaneous ovulator and non-seasonal monoestrous species (figure 2), meaning that she typically cycles once or twice per year regardless of day length, and ovulation occurs without the need for mating stimulation (Concannon, 2011; Concannon et al., 2009). The canine estrous cycle is long compared to other domestic species, with a full cycle lasting an average of 6–8 months, though considerable individual and breed variation exists (Concannon, 2011; Feldman & Nelson, 2015).

Proestrus marks the beginning of visible estrous activity and lasts an average of 6–11 days (range 0–27 days). During this phase, follicles on the ovaries mature under the influence of rising FSH and LH concentrations, and estrogen levels increase dramatically, reaching peak concentrations (50–100 pg/mL) toward the end of proestrus (Concannon, 2011; Feldman & Nelson, 2015). Estrogen induces marked proliferation and cornification of the vaginal epithelium, vulvar swelling, and a serosanguineous to hemorrhagic vaginal discharge caused by diapedesis of red blood cells through the thickening endometrium (Concannon, 2011; Johnston et al., 2001). Behaviorally, the bitch becomes attractive to males but is not yet receptive to mating, often showing playful or evasive responses to male advances (Concannon, 2011; Johnston et al., 2001).

Estrus follows proestrus and lasts 5–9 days on average (range 3–21 days). This is the period during which the bitch will stand and accept mating (Concannon, 2011; Feldman & Nelson, 2015). The transition from proestrus to estrus is marked by a sharp preovulatory surge in LH, which lasts approximately 24–48 hours and triggers ovulation 48–60 hours later (Concannon, 2011; Concannon et al., 2009). Unlike most mammals, canine ovulation releases immature oocytes that require an additional 2–3 days of maturation within the oviduct before they are capable of fertilization (Concannon, 2011; Feldman & Nelson, 2015).

Estrogen concentrations decline rapidly after the LH peak, while progesterone begins to rise even before ovulation, reaching 2–4 ng/mL at the time of the LH surge and continuing to increase thereafter (Concannon, 2011; Feldman & Nelson, 2015). This preovulatory rise in progesterone is unique to the dog and is responsible for behavioral receptivity and changes in vaginal cytology, including a shift from fully cornified to increasingly non-cornified epithelial cells (Concannon, 2011).

Diestrus (also called metestrus or the luteal phase) is the longest phase in non-pregnant bitches, lasting approximately 60–90 days (Concannon, 2011; Feldman & Nelson, 2015). During this phase, corpora lutea form from ovulated follicles and secrete progesterone, which rises to peak concentrations of 15–80 ng/mL around 15–30 days post-ovulation before gradually declining (Concannon, 2011; Feldman & Nelson, 2015).

Progesterone remains elevated for the entire diestrous period regardless of whether the bitch is pregnant, making the hormonal profile of pregnancy and pseudopregnancy nearly identical for the first 60 days (Concannon, 2011; Johnston et al., 2001). This obligatory luteal phase is maintained by pituitary secretion of both LH and prolactin, which exert luteotrophic effects on the corpus luteum (Concannon, 2011; Concannon et al., 2009).

The prolonged exposure to progesterone during diestrus has profound effects on the uterus: it stimulates endometrial gland proliferation and secretion, reduces myometrial contractility, induces cervical closure, and suppresses local immune responses, thereby creating ideal conditions for bacterial colonization and proliferation if pathogens gain entry during the preceding estrus (Hagman, 2022; Pretzer, 2008). This is why pyometra typically develops during or shortly after diestrus (Hagman, 2022; Pretzer, 2008).

Anestrus is the period of sexual and hormonal quiescence between the end of diestrus and the onset of the next proestrus. It lasts an average of 4–5 months but can range from 1 to 10 months depending on breed, age, health status, and environmental factors (Concannon, 2011; Feldman & Nelson, 2015). During anestrus, progesterone levels return to baseline (<1 ng/mL), the uterus undergoes involution (structural repair and return to normal size), and the reproductive system rests (Concannon, 2011; Johnston et al., 2001). Toward the end of anestrus, GnRH pulsatility increases, stimulating a gradual rise in FSH and LH that initiates follicular recruitment and the next proestrus (Concannon, 2011; Concannon et al., 2009).

In contrast to the bitch, the queen is a seasonally polyestrous and induced ovulator (Little, 2011; Lawler & Monti, 1984). This means that queens undergo repeated estrous cycles during a defined breeding season, and ovulation occurs only in response to the mechanical and neuroendocrine stimulus of mating (Little, 2011; Johnson, 2022) (figure 2).

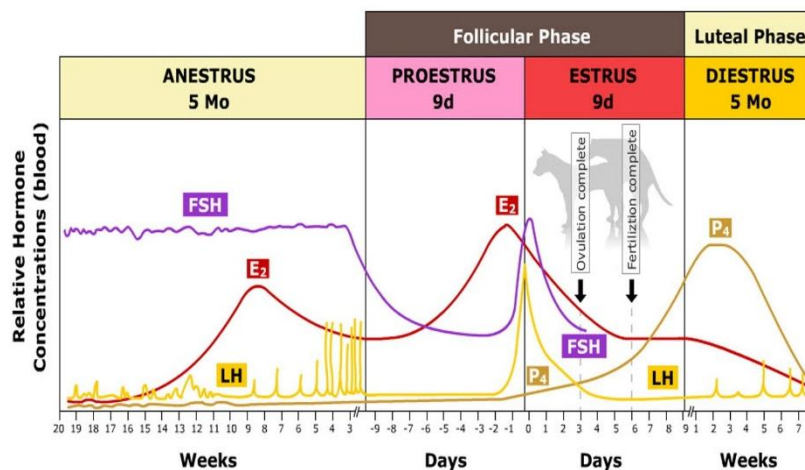


Figure 2-Reproductive cycle of a bitch

Seasonality in the queen is strongly influenced by photoperiod. Cats are considered "long-day breeders," meaning that increasing daylight hours stimulate reproductive activity (Little, 2011; Johnson, 2022). In the Northern Hemisphere, the breeding season typically begins in January or February, peaks in spring (February–April), and continues until late autumn (October–November) (Little, 2011; Lawler & Monti, 1984). Cats living near the equator or housed indoors with artificial lighting may cycle year-round, although pregnancy rates may still decrease slightly during winter months even with supplemental lighting (Little, 2011; Johnson, 2022).

Proestrus in queens is very brief, lasting 1–2 days, and is often not noticed by owners (Little, 2011; Johnson, 2022). During this phase, estrogen begins to rise, and queens may show mild behavioral changes such as increased affection or restlessness, but they do not yet accept mating (Little, 2011; Johnson, 2022).

Estrus typically lasts 5–7 days (range 2–19 days) and is characterized by pronounced behavioral changes: vocalization (loud calling), rubbing against objects and people, rolling on the floor, treading with the hindlimbs, and assuming a lordotic posture (tail deviation and elevation of the hindquarters) when the perineum is stimulated (Little, 2011; Johnson, 2022). The queen will actively solicit and accept mating during this period (Little, 2011; Johnson, 2022).

Unlike the spontaneous ovulation seen in dogs, queens require copulation to trigger ovulation. The mechanical stimulation of mating induces a surge in LH within 15 minutes to 2 hours of coitus, and ovulation occurs approximately 24–48 hours after the LH surge (Little, 2011; Lawler & Monti, 1984). Multiple matings are usually required to achieve sufficient LH release for reliable ovulation; most queens need at least 3–4 copulations within a 24-hour period (Little, 2011; Lawler & Monti, 1984). If adequate mating stimulus does not occur, LH release may be insufficient and ovulation will not take place (Little, 2011).

If the queen does not ovulate (due to absence of mating or insufficient mating stimulus), she enters interestrus, a brief period (8–9 days on average) during which estrogen declines and estrous behavior ceases (Little, 2011; Johnson, 2022). During interestrus, the previous cohort of follicles undergoes atresia and a new follicular wave begins to develop, leading the queen back into proestrus and then estrus within 1–3 weeks (Little, 2011; Johnson, 2022). This cycle of estrus and interestrus repeats throughout the breeding season until ovulation is induced or the photoperiod shortens and seasonal anestrus begins (Little, 2011; Lawler & Monti, 1984).

If ovulation is induced by mating, the queen enters diestrus (the luteal phase). Corpora lutea forms and begin secreting progesterone, which rises within 24–50 hours after the LH surge and peaks around day 21 post-mating (reaching approximately 15–30 nmol/L or 5–10 ng/mL) (Little, 2011; Johnson, 2022).

The duration and outcome of diestrus depend on whether fertilization and pregnancy occur. In non-pregnant queens (pseudopregnancy), progesterone declines to basal levels by approximately 40–45 days post-ovulation, and the queen returns to estrus (Little, 2011; Johnson, 2022). In pregnant queens, progesterone remains elevated throughout the 63–65 day gestation period and is essential for maintaining pregnancy (Little, 2011; Johnson, 2022). Unlike some species, the feline placenta has limited steroidogenic capacity, so ovarian progesterone is required until near gestation term (Little, 2011).

During anestrus, no follicular development or estrous behavior occurs, and the reproductive system rests until increasing day length in late winter triggers the resumption of cyclicity (Little, 2011).

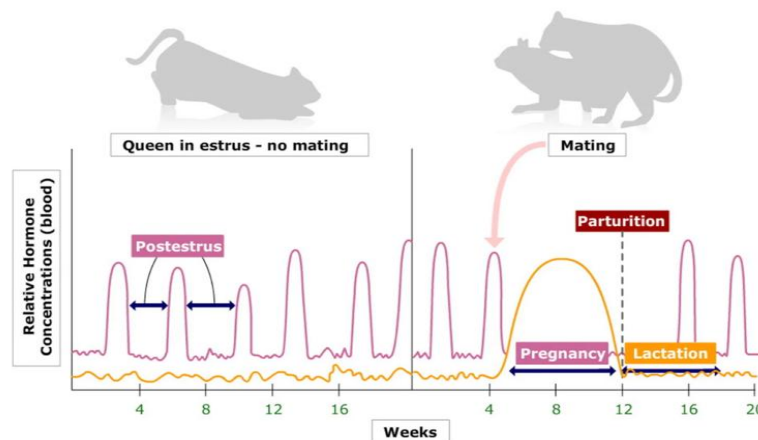


Figure 3- Reproductive cycle of a queen

The divergent reproductive strategies of bitches and queens have significant implications for disease predisposition and clinical management.

In bitches, the obligatory, prolonged luteal phase (60–90 days) exposes the uterus to high progesterone levels twice per year regardless of mating or pregnancy. This repeated progesterone exposure is the primary predisposing factor for cystic endometrial hyperplasia (CEH) and pyometra, especially in older, intact females that have undergone multiple cycles without pregnancy (Hagman, 2022; Pretzer, 2008). The long diestrus also predisposes bitches to pseudopregnancy (false pregnancy) with associated mammary development and behavioral changes (Johnston et al., 2001; Concannon, 2011).

In queens, the induced nature of ovulation and shorter, conditional luteal phase mean that progesterone exposure occurs only after mating and is of shorter duration (40–45 days in pseudopregnancy, 63–65 days in pregnancy) (Little, 2011; Johnson, 2022). As a result, pyometra is significantly less common in cats than in dogs (Hagman, 2022; Little, 2011). However, queens that cycle repeatedly without mating or pregnancy are exposed to prolonged and repeated estrogenic stimulation, which may predispose to persistent estrus behavior, ovarian cysts, and, over time, endometrial pathology (Verstegen et al., 2008; Little, 2011).

1.2 PYOMETRA

Pyometra is a serious and potentially life-threatening bacterial infection of the uterus, characterized by the accumulation of purulent material (pus) within the uterine lumen. It occurs predominantly in intact bitches and queens during or shortly after diestrus, when progesterone-mediated changes in uterine physiology and local immunity create favorable conditions for bacterial colonization and proliferation (Pretzer, 2008; Hagman, 2022; Xavier et al., 2023). The disease results from a complex interplay of hormonal, anatomical, immunological, and microbiological factors and remains one of the most common reproductive disorders in middle-aged to older, non-spayed female dogs and cats (Veiga et al., 2017; Hagman, 2022; Xavier et al., 2023).

The pathogenesis of pyometra is intimately linked to the endocrine environment of diestrus. Following estrus, progesterone concentrations rise and remain elevated for 60–90 days in bitches, regardless of whether pregnancy occurs (Pretzer, 2008; Concannon, 2011). This obligatory and prolonged luteal phase stimulates endometrial gland proliferation and secretion, reduces myometrial contractility, induces cervical closure, and suppresses local immune responses—particularly neutrophil migration and phagocytic activity—thereby impairing uterine defense mechanisms against ascending bacteria (Pretzer, 2008; Veiga et al., 2017; Hagman, 2022).

Bacterial colonization of the uterus typically occurs via ascending infection from the vagina during or shortly after estrus, when the cervix is temporarily relaxed (Hagman, 2022; Xavier et al., 2023). *Escherichia coli* is isolated in 58–90% of canine and feline pyometra cases and is the most frequently identified pathogen (Hagman, 2022; Xavier et al., 2023; Camozzi et al., 2023). Importantly, *E. coli* is also a component of the normal commensal vaginal microbiota in healthy bitches, with prevalence rates of 38–44% reported in culture-based studies (Leps et al., 2024; Schäfer-Somi et al., 2024). The *E. coli* strains associated with pyometra, however, often possess extraintestinal pathogenic virulence factors—including adhesins (papA, sfaS), toxins (hlyD, cnf1), and iron-acquisition systems (iutA)—that enhance their capacity to colonize and damage the uterine epithelium (Xavier et al., 2020; Hagman, 2022). Other bacterial species, such as *Staphylococcus* spp., *Streptococcus* spp., *Proteus* spp., and *Pseudomonas* spp., are also isolated, either alone or in mixed cultures, particularly in feline pyometra (Hagman, 2022; Camozzi et al., 2023; Xavier et al., 2023).

Once established within the hormonally primed uterus, bacterial proliferation triggers an intense local and systemic inflammatory response. Bacterial endotoxins, particularly lipopolysaccharides (LPS) from Gram-negative organisms, breach the endometrial barrier and enter the systemic circulation, provoking cytokine release, endothelial activation, and systemic inflammatory response syndrome (SIRS) (Hagman, 2022; Xavier et al., 2022, 2023). Clinically, SIRS manifests as leukocytosis or leukopenia, fever or hypothermia, azotemia, elevated liver enzymes, electrolyte disturbances, and, in severe

Pyometra is classified into two clinical forms based on cervical patency (Hagman, 2022; Xavier et al., 2023). Open-cervix pyometra occurs when the cervix remains partially dilated, allowing purulent or serosanguineous vaginal discharge to drain externally; this form is typically recognized earlier by owners due to the visible discharge. Closed-cervix pyometra is more critical, as the sealed cervix prevents drainage, leading to rapid accumulation of pus, marked uterine distension, and increased risk of uterine rupture, peritonitis, septicemia, and death (Hagman, 2022; Paudel et al., 2023; Xavier et al., 2023).

Histopathologically, pyometra is associated with variable degrees of endometrial pathology, ranging from CEH with mild inflammation to severe suppurative endometritis, interstitial fibrosis, and, in chronic cases, endometrial atrophy (Hagman, 2022; Veiga et al., 2017).

1.2.1 Risk Factors

Pyometra is a multifactorial disease, and its occurrence is influenced by the interaction of multiple host, hormonal, and environmental factors (Hagman, 2022; Xavier et al., 2023). While the fundamental pathogenic mechanism—progesterone-induced endometrial change coupled with ascending bacterial infection—is similar across bitches and queens, certain risk factors exhibit species-specific patterns of prevalence and significance (Hagman, 2022).

Pyometra predominantly affects middle-aged to older intact females. In bitches, the average age at diagnosis is approximately 7–9 years, with peak incidence occurring between 7 and 12 years (Smith, 2006; Xavier et al., 2023). However, pyometra has been documented in animals ranging from 4 months to 20 years of age, particularly in cases where exogenous hormones have been administered (Hollinshead & Verstegen, 2016; Xavier et al., 2023). In queens, the median age at presentation is similar, with most cases occurring after 5–7 years of age (Hollinshead & Verstegen, 2016).

The use of progestogens (synthetic progestins) or estrogens for reproductive control or mating prevention significantly elevates the risk of pyometra in both dogs and cats (Bhatti et al., 2007; Romagnoli, 2015; Xavier et al., 2023).

Progestogen-based contraceptives, such as medroxyprogesterone acetate (MPA), megestrol acetate (MA), and proligestone (PRG), mimic the effects of endogenous progesterone but with substantially greater potency and prolonged duration of action, thereby inducing or exacerbating CEH, suppressing uterine immunity, and promoting bacterial colonization (Bhatti et al., 2007; Romagnoli, 2015).

In bitches, administration of estrogens (particularly estradiol cypionate) for termination of unwanted pregnancies has been associated with a 25% incidence of subsequent pyometra, particularly in young bitches (1–4 years of age), and is now contraindicated in most clinical settings (Smith, 2006; Feldman & Nelson, 2015). In queens, progestin use has been linked to an increased risk of mammary tumors and pyometra, especially when administered at higher doses or outside of anestrus (Romagnoli, 2015). Lower-dose proligestone protocols appear to carry a reduced risk compared to higher-dose megestrol acetate, but progestin use remains generally discouraged in modern small-animal practice due to the availability of safer, permanent surgical sterilization (Romagnoli, 2015).

Obesity has been identified as a potential risk factor for pyometra, possibly due to altered steroid hormone metabolism, chronic low-grade inflammation, and insulin resistance, all of which may influence endometrial physiology and immune function (Hagman, 2022). However, the independent contribution of obesity to pyometra risk, when controlling for age and reproductive history, has not been conclusively established (Hagman, 2022).

Certain dog breeds exhibit elevated incidence rates of pyometra, suggesting a genetic or breed-specific predisposition. Breeds reported to be at increased risk include the Rottweiler, Golden Retriever, Rough Collie, Saint Bernard, Bernese Mountain Dog, English Springer Spaniel, Miniature Schnauzer, and Cavalier King Charles Spaniel (Egenvall et al., 2001; Jitpean et al., 2012). The mechanisms underlying breed predisposition are not fully understood but may involve inherited differences in progesterone receptor sensitivity, endometrial response to hormonal stimulation, or local immune function (Hagman, 2022).

In cats, breed predisposition has been less extensively studied, but recent evidence suggests that certain purebreeds—including Siberians, Ocicats, Korats, Siamese, Ragdolls, Maine Coons, Burmese, Birmans, and Bengals—may be at higher risk than domestic shorthair cats (Hollinshead & Verstegen, 2016).

1.2.2 Clinical and Clinicopathological Signs

Pyometra usually affects intact, middle-aged to older bitches and queens and often develops several weeks after estrus, when owners may no longer associate clinical signs with the preceding heat (Smith, 2006; Hagman, 2012). The clinical presentation ranges from mild, non-specific signs to fulminant septic shock and is strongly influenced by cervical patency and the patient's systemic response (Verstegen et al., 2008; Hollinshead & Krekeler, 2016).

In bitches with open-cervix pyometra, a mucopurulent, purulent, or sanguinopurulent vaginal discharge is a hallmark finding and may be continuous or intermittent, with colour and consistency varying according to the causative bacteria (Hagman et al., 2006; Smith, 2006; Pretzer, 2008). Additional common signs include lethargy, reduced appetite, vomiting, polyuria and polydipsia (PU/PD), dehydration and abdominal distension, and many affected bitches show clear evidence of systemic compromise at presentation (Jitpean et al., 2017; Bergström, 2017). In queens, vulvar discharge, anorexia, and lethargy are also frequently reported, but PU/PD appears to be a less consistent feature than in dogs (Agudelo, 2005; Hollinshead & Krekeler, 2016; Hagman, 2018). Because cats are meticulous groomers, owners may not notice the discharge, and some queens present only with vague signs such as depression, weight loss, and decreased appetite, making diagnosis more challenging (Kenney et al., 1987; Hollinshead & Krekeler, 2016). In both species, patients with closed-cervix pyometra lack vulvar discharge and instead exhibit signs of systemic inflammatory response, such as marked lethargy, vomiting, tachycardia, tachypnoea, pale or congested mucous membranes and variable pyrexia or hypothermia (Hagman, 2012; Jitpean et al., 2017).

Physical examination may reveal vulvar discharge in open-cervix cases, as well as dehydration, poor body condition, abdominal pain and, in some dogs and cats, palpably enlarged uterine horns (Hollinshead & Krekeler, 2016; Bergström, 2017). In closed-cervix pyometra, a tense, fluid-filled tubular structure may be palpated in the caudal abdomen, reflecting uterine distension by purulent material and indicating an increased risk of uterine rupture (Hollinshead & Krekeler, 2016; Jitpean et al., 2017). In open-cervix cases, especially in queens, uterine distension may be minimal and only a thickened uterus can be palpated, so careful abdominal palpation and thorough vaginal inspection are essential (Agudelo, 2005; Hollinshead & Krekeler, 2016).

Haematology and serum biochemistry provide important supportive evidence and help assess disease severity and systemic involvement, although results can be within reference intervals in a subset of animals (Fransson et al., 1997; Verstegen et al., 2008). Leukocytosis with neutrophilia and a left shift is a common finding in both species, but a normal leukogram does not exclude pyometra (Fransson et al., 1997; Hollinshead & Krekeler, 2016).

A normocytic, normochromic, non-regenerative anaemia consistent with chronic inflammation is frequently observed in bitches and reflects decreased erythropoiesis and bone marrow suppression (Verstegen et al., 2008; Bergström, 2017). Leukopenia occurs less often and is usually associated with severe sepsis or septic shock (Børresen, 1980). After OVH, haematological parameters generally improve in parallel with clinical status (Bartoskova et al., 2007).

On serum biochemistry, increased alkaline phosphatase (ALP) activity is reported as the most consistent abnormality in bitches with pyometra and is also described in a proportion of queens (Agudelo, 2005; Verstegen et al., 2008; Hagman, 2018). Hypercholesterolaemia and hyperbilirubinaemia can occur and are usually attributed to intrahepatic cholestasis secondary to systemic inflammation and endotoxemia (Børresen & Skrede, 1980). Hypoalbuminaemia, often accompanied by hyperproteinaemia and hyperglobulinaemia, is common in dogs and cats with pyometra and sepsis and reflects a combination of negative acute-phase response, increased vascular permeability and chronic antigenic stimulation (Deysine & Stein, 1980; Agudelo, 2005; Maddens et al., 2011). In advanced cases with sepsis and shock, hypoglycaemia may develop due to increased peripheral glucose consumption and impaired gluconeogenesis (Agudelo, 2005). Evaluation of renal parameters is crucial, as pre-renal azotemia from dehydration or intrinsic renal injury due to endotoxemia and septic nephropathy are relatively frequent and influence fluid and antimicrobial choices (Hagman, 2012; Bergström, 2017).

1.2.3 Diagnosis

Diagnostic confirmation of pyometra is based on the combination of history, clinical signs, laboratory changes and imaging findings in entire bitches and queens. Suspicion arises in an intact female with recent estrus that develops systemic illness (lethargy, anorexia, vomiting, abdominal pain) with or without vulvar discharge, and is then strengthened by inflammatory haematology/biochemistry profiles and evidence of an enlarged, fluid-filled uterus on abdominal imaging, particularly ultrasonography (Smith, 2006; Verstegen et al., 2008). Hagman, 2012).

Ultrasonography is the imaging modality of choice for diagnosing pyometra and differentiating it from other causes of uterine enlargement, such as pregnancy, mucometra, hydrometra and uterine neoplasia (Smith, 2006; Hagman, 2012). In non-pregnant bitches the normal uterus is often inconspicuous, appearing as a thin tubular structure in the caudal abdomen, between the urinary bladder and descending colon, and its size and echogenicity vary with body size, estrous stage, parity, and the presence of uterine disease (Smith, 2006; Nyland & Mattoon, 2015).

In pyometra, the uterine horns are typically enlarged, usually in a fairly symmetrical fashion, although segmental distension or unilateral involvement can occur, and the lumen contains easily visualized fluid that ranges from anechoic or hypoechoic to more heterogeneous with swirling echogenic particulate material, reflecting purulent exudate; Even slight increases in uterine diameter are sufficient to detect and characterize the intraluminal contents using ultrasound to detect and characterize intraluminal content (Smith, 2006; Verstegen et al., 2008). Echogenicity and distribution of this fluid can support differentiation between hydrometra, mucometra, and pyometra, but are not pathognomonic and must be interpreted together with the clinical picture and laboratory findings (Hagman, 2012; Nyland & Mattoon, 2015).

The uterine wall in pyometra may appear variably thickened, sometimes with segmental variation and cystic changes suggestive of concurrent cystic endometrial hyperplasia, or markedly thinned when distension is severe, regardless of the nature of the luminal fluid (De Bosschere et al., 2001; Schlafer & Gifford, 2008). Atypical forms, such as emphysematous pyometra, show gas within the uterine lumen; ultrasonographically this appears as a gas-filled tubular structure lacking the typical layered wall pattern and peristalsis of the small intestine, and continuity with the ovary or uterine stump helps distinguish it from intestinal loops (Feliciano et al., 2013). Uterine stump pyometra is seen as a blind-ending, fluid-filled sac between the bladder and descending colon, and identification of concurrent ovarian remnants or follicular structures supports the diagnosis of ovarian remnant syndrome (Johnston et al., 2001; Nyland & Mattoon, 2015). In addition, abdominal ultrasound can detect concurrent ovarian lesions (e.g. cysts, neoplasia) and signs of peritonitis, which are important for prognosis and therapeutic decision-making (Hagman, 2012; Veiga et al., 2017).

Survey radiography is less sensitive than ultrasonography but can still support the diagnosis by demonstrating generalized uterine enlargement and by helping to rule out other abdominal conditions (Smith, 2006). In non-pregnant bitches, a normal uterus is usually not visible on plain radiographs, so visualization of soft-tissue opaque tubular structures in the caudoventral abdomen suggests significant distension by fluid or soft tissue, as occurs in pyometra, mucometra, hydrometra, early pregnancy, or uterine torsion (Thrall, 2013; Smith, 2006).

Definitive aetiological diagnosis relies on microbiological evaluation of uterine or vaginal samples. Aerobic culture and AST are recommended to identify the causative organisms, most commonly *E. coli*, and to guide targeted antimicrobial therapy, although empirical treatment must be initiated before culture results are available in most clinical situations (Hagman & Greko, 2005; Verstegen et al., 2008). Blood cultures can be considered in patients with suspected bacteraemia or septic shock but are not performed routinely in all cases (Hagman, 2012; Jitpean et al., 2017).

Histopathological examination of the uterus removed at OVH typically reveals cystic endometrial hyperplasia with varying degrees of suppurative endometritis, confirming the CEH pyometra complex and providing information on the chronicity and severity of endometrial pathology (De Bosschere et al., 2001; Schlafer & Gifford, 2008).

1.2.4 Treatment

Pyometra is a medical and surgical emergency in both bitches and queens and requires rapid, decisive intervention to prevent progression to septicemia, endotoxemia and multi-organ failure (Hagman, 2012; Fieni et al., 2014). The main therapeutic goals are removal or resolution of the uterine source of infection, stabilization of systemic status, and appropriate antimicrobial coverage directed against the most likely pathogens, predominantly *E. coli* (Hagman & Greko, 2005; Verstegen et al., 2008).

Regardless of the chosen definitive treatment, initial management should include prompt intravenous fluid therapy, correction of electrolyte and acid–base imbalances, analgesia, and early institution of broad-spectrum bactericidal antibiotics, as exclusive medical treatment with antimicrobials is insufficient to resolve the disease (Hagman, 2012; Fieni et al., 2014).

OVH with removal of the entire uterus and cervix remains the safest and most effective treatment option for the majority of patients, as it eliminates the primary focus of infection and immediately interrupts systemic exposure to endotoxins and inflammatory mediators (Smith, 2006; Fieni et al., 2014). Surgical treatment is particularly recommended in older, non-breeding animals, in patients with closed-cervix pyometra, and in any case presenting with systemic illness or organ dysfunction (Hagman, 2012; Fieni et al., 2014). Perioperative antimicrobial therapy should ideally be guided by bacterial culture and AST of uterine or vaginal samples, but empirical treatment must be initiated before results are available and subsequently adjusted once AST data are obtained (Hagman & Greko, 2005; Hagman, 2018).

Because most isolates are *E. coli*, empirical protocols commonly employ beta-lactams such as amoxicillin, amoxicillin–clavulanate or ampicillin, often in combination with a fluoroquinolone (e.g. enrofloxacin or marbofloxacin), which achieves high tissue concentrations in the uterus, exceeding plasma levels (Hagman & Greko, 2005; Verstegen et al., 2008). Alternative effective options include cephalosporins and potentiated sulfonamides (sulfamethoxazole–trimethoprim), and metronidazole may be added when sepsis or mixed aerobic–anaerobic infection is suspected (Hagman & Greko, 2005; Fieni et al., 2014). Antimicrobials should be chosen to avoid nephrotoxicity, particularly in animals with concurrent azotemia, and administered intravenously in the initial phase, transitioning to oral formulations as the patient stabilizes (Hagman, 2012; Hagman, 2018).

Medical management is reserved for carefully selected patients and is associated with a higher risk of treatment failure and recurrence, which may reach 5–70% depending on protocol and follow-up period (Niskanen & Thrusfield, 1998; Bergström, 2017). It is primarily considered in young bitches intended for breeding (generally under 6 years of age) or queens under approximately 8 years of age, that are systemically stable, without evidence of uterine rupture or advanced systemic disease, and without severe cystic endometrial hyperplasia on imaging or histology (Hollinshead & Krekeler, 2016; Bergström, 2017). Even in these cases, meticulous monitoring is essential, and owners must be informed that subsequent OVH remains the treatment of choice if medical therapy fails or if pyometra recurs (Hollinshead & Krekeler, 2016; Bergström, 2017).

Hormonal medical treatment aims to reverse progesterone's effects on the uterus and to promote evacuation of purulent contents. Natural prostaglandin F2 α (dinoprost tromethamine) and synthetic analogues such as cloprostenol have luteolytic activity, shortening the lifespan of the corpus luteum and thereby reducing circulating progesterone; they also increase myometrial contractility and induce cervical relaxation, facilitating uterine drainage (Fieni et al., 2001; Fieni et al., 2014). Because these drugs can cause intense uterine contractions and systemic side effects (e.g. vomiting, diarrhea, abdominal pain, bronchoconstriction), they are generally reserved for open-cervix pyometra and are administered in gradually increasing doses with close clinical observation (Verstegen et al., 2008; Fieni et al., 2014). Their efficacy can be enhanced by combining them with antiprogestins such as aglepristone, a competitive progesterone-receptor antagonist that rapidly blocks progesterone action at the uterine level, leading to cervical opening, decreased glandular secretion and increased myometrial responsiveness (Fieni et al., 2001; Fieni et al., 2014). Dopamine agonists, including cabergoline and bromocriptine, are sometimes added to suppress prolactin, which exerts a luteotrophic effect in the bitch, thereby reinforcing luteolysis and accelerating progesterone decline (Verstegen et al., 2008; Fieni et al., 2014).

Throughout medical management, systemic support and antimicrobial therapy are mandatory, following the same principles as in surgically treated cases, because hormonal manipulation alone does not eradicate the intra-uterine infection (Hagman, 2012; Fieni et al., 2014). Given the substantial likelihood of recurrence, many authors recommend breeding the bitch or queen at the first estrus following successful medical treatment, as pregnancy and postpartum uterine involution have been associated with a reduced risk of subsequent uterine disease (Niskanen & Thrusfield, 1998; Bergström, 2017).

Ultimately, the choice between surgical and medical treatment must be individualized, weighing the reproductive value of the animal, the severity of systemic illness, owner expectations and resources, and the inherent risk of recurrence, but OVH remains the gold standard for definitive cure and prevention of future pyometra episodes (Smith, 2006; Hagman, 2018).

1.3 VAGINAL AND UTERINE MICROBIOTA

The vaginal and uterine microbiota of healthy female dogs and cats constitute a complex microbial ecosystem that play crucial roles in reproductive health. Unlike humans, where *Lactobacillus* species typically dominate the vaginal microbiome (90-95%), the reproductive microbiota of dogs and cats exhibits considerably greater bacterial diversity and distinct compositional patterns (Banchi et al., 2024; Hutchins et al., 2013).

In healthy bitches, the vaginal microbiota is characterized by high species richness and diversity, with the predominant bacterial phyla including Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria (Leps et al., 2025; Miller et al., 2019). At the genus level, *Escherichia-Shigella*, *Mycoplasma*, *Streptococcus*, *Staphylococcus*, and *Pasteurella* are frequently identified, regardless of reproductive state (Leps et al., 2025). Culture-based studies have shown that coagulase-positive *Staphylococcus* species, beta-hemolytic *Streptococcus* species, *E. coli*, and *Pasteurella spp.* constitute the most isolated bacteria from canine vaginal swabs (Leps-Sunnen et al., 2024).

Importantly, the prevalence of *E. coli* in the vagina of healthy bitches ranges from 38% to 44%, indicating that this potential uropathogens can be part of the normal commensal flora (Clemetson & Ward, 1990; Leps et al., 2025). This observation has significant clinical implications, as the mere isolation of bacteria such as *E. coli* from vaginal cultures does not necessarily indicate pathology or warrant antimicrobial treatment (Leps et al., 2025; Miller et al., 2019).

While *Lactobacillus* species have been identified in canine vaginal samples, they are not dominant within the canine vaginal microbiome, comprising only a small proportion of the total bacterial community (Hutchins et al., 2013; Leps et al., 2025). Studies investigating lactic acid-producing bacteria (LAB) in healthy bitches reported that LAB were recovered from approximately 60% of animals, with *Lactococcus* species being the most frequently isolated (51%), followed by *Lactobacillus* species (25%) (Otero et al., 2022; Zona et al., 2008). The vaginal pH in healthy dogs typically ranges from 6.0 to 7.5, considerably higher than the acidic pH observed in humans, where lactobacilli predominate (Zona et al., 2008).

The feline vaginal microbiota demonstrates substantial diversity. Recent metataxonomic analyses revealed that the vaginal microbiome of healthy queens is dominated by Proteobacteria, Firmicutes, Bacteroidota, and Actinobacteria at the phylum level (Banchi et al., 2024). At the genus level, *Escherichia-Shigella*, *Streptococcus*, and *Pasteurella* represent the most abundant genera in feline vaginal samples (Banchi et al., 2024).

Culture-based studies have consistently demonstrated that coagulase-negative *Staphylococcus* species, *S. canis*, and *E. coli* are the most common aerobic organisms isolated from the vagina of healthy cats, recovered from 56%, 52%, and 44% of vaginal samples, respectively (Clemetson & Ward, 1990). Mixed bacterial cultures containing one to eight different species (mean of three species) are commonly observed, with the number of colony-forming units tending to vary inversely with the number of bacterial species detected (Clemetson & Ward, 1990). Additional bacteria frequently isolated from healthy feline vaginal samples include *Corynebacterium* species, *Haemophilus* species, *Klebsiella* species, and *Pseudomonas aeruginosa* (Maksimovic et al., 2023; Termelioğlu et al., 2022).

Anaerobic bacteria are less frequently detected in the healthy feline vagina, being identified in approximately 13% of the samples, and included species such as *Peptostreptococcus*, *Bacteroides*, and *Lactobacillus* (Clemetson & Ward, 1990; Termelioğlu et al., 2022). Notably, no significant differences in vaginal bacterial flora composition have been observed between household and stray cats, or across different stages of the estrous cycle, suggesting relative stability of the commensal microbiota (Banchi et al., 2024; Maksimovic et al., 2023).

In healthy non-pregnant cats, uterine bacterial cultures are typically negative, with bacteria isolated from only two of 29 uterine samples in one study, compared to 52 of 53 positive vaginal cultures from the same animals (Clemetson & Ward, 1990; Maksimovic et al., 2023). This stark contrast between vaginal and uterine bacterial loads underscores the effectiveness of cervical barrier mechanisms in preventing ascending bacterial colonization under physiological conditions (Maksimovic et al., 2023).

The recognition that the reproductive tract harbors diverse bacterial communities, including potential pathogens as part of the normal commensal flora, has profound implications for clinical practice. The presence of bacteria such as *E. coli*, *Staphylococcus*, or *Streptococcus* in vaginal cultures from asymptomatic animals does not necessarily indicate infection and should not automatically trigger antimicrobial therapy (Leps et al., 2025; Miller et al., 2019). Antimicrobial treatment should only be considered when positive uterine cultures are accompanied by clinical signs of inflammation or disease are identified through a comprehensive gynecological examination (Leps-Sunnen et al., 2024).

Reproductive hormones, particularly estrogen and progesterone, influence the composition, diversity, and functional characteristics of the vaginal and uterine microbiota in dogs and cats (Chen et al., 2021; Moreno & Franasiak, 2017).

Estrogen plays a multifaceted role in shaping the reproductive microbiome during the follicular phase and estrus (Hagman, 2022; Verstegen et al., 2008).

Estrogen influences vaginal microbial composition through modulation of epithelial cell metabolism and immune responses. (Hagman, 2022; Verstegen et al., 2008).

Progesterone induces CEH, characterized by thickening of the endometrial lining, proliferation of endometrial glands, and increased secretion of nutrient-rich fluids into the uterine lumen (Hagman, 2022; Nascimento et al., 2024). This creates an ideal growth medium for bacteria that have ascended during estrus (Verstegen et al., 2008).

Progesterone causes cervical closure and mucus thickening, preventing drainage of uterine contents and bacterial clearance (Davidson et al., 2016; Nascimento et al., 2024). Myometrial contractility is markedly reduced under progesterone influence, further impairing the uterus's mechanical ability to expel bacteria (Hagman, 2022; Verstegen et al., 2008).

1.3.1 Bacteriology and Microbiological Sampling

Proper sample collection represents a critical step in microbiological diagnosis of pyometra, as the quality of bacteriological results depends entirely on obtaining representative specimens while minimizing contamination from commensal vaginal flora. The timing of sample collection, technique employed, and handling procedures profoundly influence culture results and subsequent antibiotic selection decisions (Paudel et al., 2023).

Samples for AST, also known as antibiotic sensitivity testing or antibiogram, should be obtained before initiating empirical antibiotic therapy, as prior antibiotic exposure may suppress bacterial growth in culture, yielding false-negative results or failing to isolate the causative pathogen (Davidson et al., 2016; Paudel et al., 2023). However, the clinical status of bitches with pyometra often requires immediate initiation of antimicrobial treatment, particularly in systemically ill patients presenting with sepsis or closed-cervix pyometra, where delaying therapy could be life-threatening (Hagman, 2018). In such cases, samples should still be collected as soon as practically feasible, even if antimicrobials have already been administered, as culture may still provide diagnostic information that enables refinement of therapy based on susceptibility results (Egenvall et al., 2024).

The gold standard method for obtaining bacteriological samples in pyometra cases, managed surgically, involves direct collection of uterine contents during OVH, immediately after removal of the infected uterus. This approach provides the most accurate representation of the causative bacteria within the uterine lumen while avoiding contamination from vaginal flora (Coggan et al., 2008; Lopes et al., 2021; Silva et al., 2021).

The technique involves using strict aseptic procedures to aspirate purulent material directly from the uterine lumen using sterile disposable syringes and needles. Typically, 3-5 mL of intrauterine content is aspirated from each uterine horn after the uterus has been exteriorized and isolated from the abdomen (Coggan et al., 2008; Wanangkarn et al., 2023). Alternatively, sterile cotton swabs can be introduced into the uterine lumen through a small incision made with sterile surgical blades, ensuring that the swab contacts only the intrauterine purulent material and not the external uterine serosa or vaginal tract (Paudel et al., 2023; Wanangkarn et al., 2023).

Some protocols also recommend collecting tissue samples from the uterine wall itself which can be cultured to detect bacteria embedded within the endometrial tissue that may not be present in free luminal fluid (Hagman et al., 2006; Wanangkarn et al., 2023). This is particularly relevant in cases where bacteria have invaded the endometrium or formed biofilms on the uterine wall (Lopes et al., 2021).

Transcervical or cranial vaginal swab sampling could represent an alternative or be a complementary collection method. Double-guarded swabs are recommended to minimize vaginal contamination during passage through the cervix into the uterine lumen (Davidson et al., 2016; Egenvall et al., 2024).

Proper handling and transport of collected samples is essential to maintain bacterial viability and prevent overgrowth of contaminating organisms. Samples should be transported to the microbiology laboratory as quickly as possible, ideally within 30 minutes to 2 hours of collection (Coggan et al., 2008; Ylhäinen et al., 2024). If immediate transport is not feasible, samples should be refrigerated at +4°C until processing, which should occur within 24 hours (Coggan et al., 2008; Ylhäinen et al., 2024).

All samples must be clearly labeled with the patient's identification, date and time of collection, anatomical site of collection (uterine content, uterine tissue, vaginal swab), and clinical history, including recent antibiotic exposure (Paudel et al., 2023). Accompanying microbiology request forms should indicate suspected diagnosis (pyometra), clinical severity, and any known antimicrobial allergies to guide appropriate susceptibility testing (Silva et al., 2021).

Different culture media are used to ensure optimal recovery and identification of the microorganisms involved. Blood agar, containing 5% sheep blood, is an enriched and non-selective medium that supports the growth of most bacterial pathogens, including fastidious species (Coggan et al., 2008; Paudel et al., 2023). It also allows observation of hemolytic activity, which provides valuable preliminary identification clues. MacConkey agar is a selective and differential medium specifically formulated for the isolation of Gram-negative enteric bacteria, commonly implicated in pyometra, while inhibiting Gram-positive growth (Allen, 2006; Coggan et al., 2008). It further distinguishes lactose-fermenting organisms, which produce pink colonies, from non-lactose fermenters that form colorless colonies (Coggan et al., 2008).

To enhance bacterial recovery, particularly when organisms are present in low numbers or have been stressed by prior antibiotic treatment, samples can also be cultured in Brain Heart Infusion (BHI) broth, a nutrient-rich liquid medium (Coggan et al., 2008). After incubation, subculturing onto solid media, such as blood agar and MacConkey agar, facilitates the isolation of individual colonies (Coggan et al., 2008).

In cases where anaerobic bacteria are suspected, samples should be incubated under anaerobic conditions using media like Brucella blood agar or Centers for Disease Control and Prevention (CDC) anaerobe blood agar. This is particularly relevant when a foul-smelling discharge is present or when aerobic cultures fail to yield bacterial growth despite clinical evidence of infection (Coggan et al., 2008).

Although rare, fungal cultures may also be indicated, especially in immunocompromised animals or those exposed to prolonged antibiotic therapy. In such cases, samples are inoculated onto Sabouraud dextrose agar and incubated at room temperature for at least seven days to detect slow-growing fungi (Coggan et al., 2008).

Given the increasing prevalence of AMR among bacterial isolates from canine and feline pyometra, empirical antimicrobial selection based solely on historical susceptibility patterns is no longer reliable (Paudel et al., 2023; Silva et al., 2021; Wanangkarn et al., 2023). Susceptibility patterns vary geographically, temporally, and between individual patients, necessitating individualized AST to ensure appropriate therapy (Egenvall et al., 2024). Inappropriate antimicrobial selection due to resistance can result in therapeutic failure, prolonged infection, increased patient morbidity and mortality, and further propagation of resistant bacterial strains (Hagman, 2018).

The Kirby-Bauer disk diffusion method represents the most widely utilized technique in veterinary clinical microbiology laboratories due to its simplicity, cost-effectiveness, and ability to test multiple antimicrobials simultaneously (Hudzicki, 2009; Silva et al., 2021).

The test is based on the principle that when an antimicrobial-impregnated paper disk is placed on an agar surface inoculated with bacteria, the antimicrobial agent diffuses radially outward from the disk into the surrounding agar, creating a concentration gradient (Hudzicki, 2009). If the bacterial isolate is susceptible to an antimicrobial, a clear circular zone devoid of visible bacterial growth (zone of inhibition) forms around the disk. The diameter of this zone correlates inversely with the minimum inhibitory concentration (MIC): larger zones indicate greater susceptibility (lower MIC), while smaller zones or no zone indicates resistance (Hudzicki, 2009; Wira et al., 2015). These measured diameters are compared to standardized interpretive criteria (breakpoints) established by organizations such as the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST), which classify isolates as Susceptible (S), Intermediate (I), or Resistant (R) (Hudzicki, 2009).

In pyometra cases, bacterial isolates are typically tested against panels of antimicrobials relevant to the treatment of Gram-negative and Gram-positive infections. Commonly tested antibiotics include penicillin, ampicillin, amoxicillin, amoxicillin-clavulanate, cephalexin, cephalothin, cefoxitin, ceftriaxone, enrofloxacin, ciprofloxacin, marbofloxacin, gentamicin, amikacin, tobramycin, neomycin, tetracycline, doxycycline, erythromycin, lincomycin, clindamycin, sulfamethoxazole-trimethoprim, sulphadiazine-trimethoprim, chloramphenicol, polymyxin B, and vancomycin (Coggan et al., 2008; Paudel et al., 2023; Silva et al., 2021).

While the disk diffusion method provides qualitative or semi-quantitative susceptibility results (S/I/R), determination of the MIC provides quantitative measurement of antimicrobial potency against a specific organism (Andrews, 2001; Mouton et al., 2018).

1.4 ANTIMICROBIAL THERAPY

The antimicrobial families most frequently used to treat pyometra in bitches and queens include β -lactams, fluoroquinolones, aminoglycosides, tetracyclines, and potentiated sulfonamides, largely because these drugs combine adequate distribution to the uterine lumen with activity against the predominant pathogen, *Escherichia coli*. Current small-animal internal medicine and infectious disease textbooks describe β -lactams as traditional first-line options, with aminopenicillins (ampicillin, amoxicillin) and aminopenicillins combined with β -lactamase inhibitors (amoxicillin–clavulanate) widely used empirically in dogs and cats presenting with open- or closed-cervix pyometra (Greene, 2022; Papich, 2021). However, several microbiological studies have documented substantial resistance of uterine *E. coli* isolates to penicillin and ampicillin in canine pyometra, which has led authors to recommend third-generation cephalosporins, such as cefotaxime or ceftiofur, for systemically ill patients until culture and susceptibility results become available (Coggan et al., 2008; Silva et al., 2021). Fluoroquinolones, particularly enrofloxacin, marbofloxacin and pradofloxacin, also show excellent in-vitro activity against Gram-negative bacilli from the reproductive tract and achieve high concentrations in uterine tissue and secretions, so they are often proposed as alternative or adjunctive agents in severe or refractory cases (Boothe, 2015; Papich, 2021). Nonetheless, recent veterinary antimicrobial-stewardship guidelines advise that fluoroquinolones should preferably be reserved for infections documented by culture and susceptibility testing, given their classification as critically important antimicrobials in human medicine (Guardabassi et al., 2018).

Aminoglycosides such as gentamicin and amikacin retain high activity against *E. coli* isolated from canine pyometra and have been associated with susceptibility rates above 80–90% in several series, making them valuable components of combination regimens in septic or hospitalized patients (Coggan et al., 2008; Paudel et al., 2023). Their use is, however, limited by the risk of nephrotoxicity and ototoxicity, particularly in older animals with dehydration or pre-existing renal dysfunction, so dosing must follow current pharmacokinetic–pharmacodynamic recommendations and, when possible, be guided by therapeutic drug monitoring (Boothe, 2015; Papich, 2021). Tetracyclines, mainly doxycycline, and potentiated sulfonamides (trimethoprim–sulfamethoxazole) have broad antibacterial spectra and good oral bioavailability, and they are sometimes employed as step-down therapy after OVH and initial parenteral treatment in stable patients; however, rising resistance among companion-animal *E. coli* isolates reduces their suitability as sole empirical agents in life-threatening pyometra (Guardabassi et al., 2018; Papich, 2021).

Although the evidence base for queens is more limited, available case series suggest that therapeutic principles parallel those described in bitches: clinicians most often choose amoxicillin–clavulanate or a second- or third-generation cephalosporin as initial therapy, occasionally combined with a fluoroquinolone in critically ill animals, while avoiding nephrotoxic aminoglycosides in cats with azotaemia or hypovolaemia (Nelson & Couto, 2020; Papich, 2021). Across both species, contemporary reviews stress that surgical removal of the infected uterus remains the cornerstone of treatment, and systemic antimicrobials should be selected empirically only for the shortest necessary period and then refined or de-escalated according to uterine culture and antimicrobial susceptibility results, in line with veterinary antimicrobial-stewardship recommendations (Guardabassi et al., 2018; Greene, 2022).

1.4.1 Antimicrobial Resistance

Antimicrobial resistance (AMR) occurs when bacteria develop mechanisms that allow them to survive exposure to antimicrobial agents that would normally inhibit their growth or kill them. Understanding these resistance mechanisms is essential to optimizing antimicrobial therapy for pyometra, as resistance profoundly impacts treatment outcomes and requires rational antimicrobial selection based on susceptibility testing rather than empirical choices alone (Gajda et al., 2018; Prestinaci et al., 2015).

Bacteria employ four major mechanisms to resist antimicrobial action: enzymatic inactivation or drug modification, alteration of the antimicrobial target site, reduction of intracellular drug accumulation through decreased permeability or increased efflux, and metabolic bypass of inhibited pathways (Blair et al., 2015; Munita & Arias, 2016; Prestinaci et al., 2015). These mechanisms may be intrinsic to a bacterial species or acquired via chromosomal mutations and, importantly, through horizontal gene transfer of resistance determinants from other bacteria (Munita & Arias, 2016).

The production of β -lactamase enzymes is the most prevalent and clinically significant resistance mechanism against β -lactam antibiotics, including penicillins, cephalosporins and carbapenems (Bush, 2018; Naas et al., 2017).

β -lactamases hydrolyses the β -lactam ring that is essential for antimicrobial activity, thereby inactivating these drugs before they can bind to their target penicillin-binding proteins (PBPs) located in the bacterial cell wall (Bush, 2018; Ghosh & Lahiri, 2021).

In addition, bacteria can develop resistance by altering the structure of antimicrobial targets, reducing drug-binding affinity while maintaining essential physiological functions (Blair et al., 2015; Munita & Arias, 2016). For β -lactam antibiotics, this typically involves mutations in, or acquisition of, genes encoding altered PBPs with reduced affinity for β -lactam drugs, which allows continued cell-wall synthesis despite the presence of the antibiotic (Munita & Arias, 2016; Sauvage et al., 2008).

Efflux pumps are membrane transport proteins that actively extrude antimicrobial agents from the bacterial cell, lowering intracellular drug concentrations below therapeutic levels (Blair et al., 2015; Du et al., 2018; Laborda et al., 2023).

These systems represent an important intrinsic resistance mechanism in Gram-negative bacteria and contribute to their comparatively reduced susceptibility to many antimicrobial classes, particularly fluoroquinolones, tetracyclines, and macrolides, when compared with Gram-positive organisms (Li et al., 2015; Piddock, 2006). Gram-negative bacteria also possess an outer membrane containing porins, water-filled channels that regulate the passage of hydrophilic molecules, including many antibiotics, into the periplasmic space (Blair et al., 2015; Delcour, 2009). Mutations leading to loss, structural alteration or reduced expression of major porins (such as OmpF and OmpC in *Escherichia coli*) decrease antimicrobial influx and thus contribute to resistance, particularly when combined with efflux pump overexpression or β -lactamase production (Delcour, 2009; Pagès et al., 2008). This porin-mediated mechanism is especially relevant for carbapenems and certain fluoroquinolones, which rely heavily on porin channels to enter Gram-negative bacterial cells (Blair et al., 2015; Pagès et al., 2008).

The antimicrobial susceptibility patterns of bacteria isolated from canine and feline pyometra have evolved substantially over recent decades, with concerns increasing in resistance to commonly used antimicrobials and emergence of MDR and extended-spectrum beta-lactamase (ESBL)-producing strains (Chen et al., 2019; Hagman & Greko, 2005; Sroithongkham et al., 2024). Recent investigations reported MDR in 26-71.15% of bacterial isolates from pyometra in small animals, indicating widespread acquisition of multiple resistance determinants (Abad-Fau et al., 2024; Paudel et al., 2023; Silva et al., 2021).

Antimicrobial resistance in feline pyometra follows similar trends to canine pyometra, with *E. coli* remaining the predominant pathogen and exhibiting increasing resistance to commonly used antimicrobials (Elmansy et al., 2025; Johnson, 2022; Potter et al., 2016).

Among β -lactam antibiotics, resistance to penicillin and ampicillin was consistently high across multiple studies, with reported rates ranging from 53.2% to 85.5% for ampicillin and even higher values for penicillin (Paudel et al., 2023; Silva et al., 2021). This high prevalence of resistance made these narrow-spectrum penicillins increasingly unreliable as empirical therapy for pyometra. Amoxicillin–clavulanate, which combines a β -lactam with a β -lactamase inhibitor, showed improved but still variable efficacy, with only a proportion of isolates remaining susceptible (Bassessar et al., 2013).

Cephalosporin susceptibility varied according to antibiotic generation. First-generation cephalosporins showed moderate resistance rates of approximately 45–55%, whereas third-generation cephalosporins generally retained better activity, although extended-spectrum β -lactamase (ESBL)-producing strains were resistant to these agents as well (Paudel et al., 2023; Sroithongkham et al., 2024). In one study, 42.3% of *E. coli* isolates from canine pyometra were resistant to third-generation cephalosporins and/or fluoroquinolones, and 14.4% were confirmed ESBL producers (Sroithongkham et al., 2024).

Fluoroquinolones were widely used in veterinary medicine to treat severe infections because of their broad spectrum of activity and excellent tissue penetration. Reported resistance rates to fluoroquinolones in pyometra isolates varied geographically but generally ranged from 4% to 15% of isolates (Hagman & Greko, 2005; Paudel et al., 2023; Silva et al., 2021). As fluoroquinolones are classified as critically important antimicrobials by the World Health Organization (WHO), prudent use and preservation of their efficacy were considered essential (WHO, 2019).

Aminoglycosides consistently showed high efficacy against bacteria isolated from pyometra. Several studies reported susceptibility rates of 85.5–100% for the most commonly used aminoglycosides and 80.6–95% for other molecules in this class (Bassessar et al., 2013; Paudel et al., 2023; Silva et al., 2021). This excellent activity made aminoglycosides valuable empirical options, particularly for severe or MDR infections, although their use required careful consideration of nephrotoxicity risk, especially in patients with pre-existing renal compromise, which was common in pyometra (Paudel et al., 2023).

Carbapenems retained exceptional activity against pyometra isolates, with susceptibility rates close to 95.2–100% (Paudel et al., 2023; Silva et al., 2021). However, because they are categorized as critically important antimicrobials of highest priority, carbapenems should be reserved as last-line agents for infections caused by MDR organisms, particularly ESBL producers, when other therapeutic options have failed or were contraindicated (WHO, 2019).

Resistance to tetracyclines was frequently observed, affecting approximately 40–80% of isolates and limiting their usefulness as empirical therapy in pyometra (Bassessar et al., 2013; Paudel et al., 2023). Lincosamides showed extremely high resistance rates, close to 96.8%, rendering them ineffective for the treatment of pyometra despite their frequent use in other veterinary infections (Paudel et al., 2023). Sulfamethoxazole–trimethoprim exhibited variable susceptibility, ranging from about 45% to 90%, depending on geographic region and study population (Bassessar et al., 2013; Hagman & Greko, 2005; Silva et al., 2021).

Antimicrobial resistance patterns exhibit substantial geographic variation, reflecting differences in antimicrobial use policies, prescribing practices, veterinary regulations, and surveillance infrastructure across regions and countries (Hagman & Greko, 2005; Paudel et al., 2023). Nordic countries with strict antimicrobial stewardship policies and prescription-only access to veterinary antimicrobials typically reported lower resistance rates compared to regions with less stringent controls (Hagman & Greko, 2005).

These geographic differences emphasize that resistance data from one region cannot be extrapolated to another, and that each veterinary practice should ideally track local antimicrobial susceptibility patterns (antibiograms) to guide empirical therapy appropriately (Paudel et al., 2023; Silva et al., 2021).

The emergence and dissemination of AMR in bacteria causing pyometra results from complex interactions between antimicrobial selection pressure, bacterial genetics, animal management practices, and broader environmental and societal factors (Guardabassi et al., 2004; Prescott, 2017; Velazquez-Meza et al., 2022).

The most significant driver of AMR is inappropriate antimicrobial use, encompassing several problematic practices, such as antibiotic use without confirmed bacterial infection, selection of an antibiotic with incorrect antimicrobial spectrum, inadequate or excessive antibiotic dosing, inappropriate duration of therapy, and failure to obtain culture and susceptibility data before treatment initiation (Guardabassi et al., 2004; Prescott, 2017; Weese et al., 2019).

In small animal veterinary practice, antimicrobials are frequently prescribed empirically without diagnostic confirmation of bacterial infection or identification of the causative organism (Guardabassi et al., 2004; Weese et al., 2019). While empirical therapy is often necessary in acute, severe infections like pyometra, where delayed treatment could be life-threatening, failure to subsequently obtain cultures and adjust therapy based on susceptibility results represents a missed opportunity for antimicrobial optimization (Egenvall et al., 2024).

The use of broad-spectrum antimicrobials when narrow-spectrum agents would suffice exerts unnecessary selection pressure on commensal bacteria, promoting resistance development in the patient's endogenous microbiota (Guardabassi et al., 2004; Prescott, 2017). Similarly, prolonged antimicrobial courses beyond the minimum effective duration and underdosing that achieves subtherapeutic tissue concentrations both contribute to resistance selection (Weese et al., 2019).

The frequent use of antimicrobials classified by WHO as critically important for human medicine, particularly third-generation cephalosporins and fluoroquinolones, in companion animal practice contributes to resistance emergence in bacteria with zoonotic potential (Guardabassi et al., 2004; Prescott, 2017; WHO, 2019). Companion animals receive frequently broad-spectrum agents, including aminopenicillins plus clavulanic acid, cephalosporins, and fluoroquinolones, often mirroring antimicrobial classes used in human medicine (Guardabassi et al., 2004; Weese, 2011).

Client non-compliance with prescribed antimicrobial regimens, including premature discontinuation when clinical signs improve, inconsistent dosing, or sharing antimicrobials between pets, creates conditions favoring resistance selection through subtherapeutic drug exposure (Guardabassi et al., 2004). Additionally, over-the-counter availability of veterinary antimicrobials in some countries enables unsupervised antimicrobial use without veterinary diagnosis or guidance, promoting inappropriate use (Guardabassi et al., 2004).

Veterinary hospitals and clinics serve as potential reservoirs and transmission sites for antimicrobial-resistant bacteria (Prescott, 2017; Weese, 2011). Hospitalized animals receiving intensive antimicrobial therapy are at increased risk for colonization with resistant organisms, which can subsequently spread to other patients, veterinary personnel, and into the community through discharged animals (Prescott, 2017).

AMR transcends individual patients, veterinary practices, and even species boundaries, representing a complex, interconnected challenge that affects human health, animal health, and environmental health simultaneously. The One Health concept provides an integrative framework for understanding and addressing AMR through coordinated, multidisciplinary efforts that recognize the intrinsic linkages between these domains (Ballash et al., 2024; Velazquez-Meza et al., 2022; WHO, 2019).

Companion animals, particularly dogs and cats, can serve as reservoirs for antimicrobial-resistant zoonotic bacteria that pose direct risks to human health (Bhat et al., 2021; Guardabassi et al., 2004; Jin et al., 2023).

Methicillin-resistant Staphylococcus pseudintermedius (MRSP) infections in pets are typically acquired from colonized human owners and can cycle back to humans, creating persistent household reservoirs (Guardabassi et al., 2004; Velazquez-Meza et al., 2022). These organisms cause skin and soft tissue infections, surgical site infections, and occasionally severe invasive infections (Bhat et al., 2021).

ESBL-producing *E. coli* and *Klebsiella* species are increasingly identified in companion animals, including in cases of pyometra and urinary tract infections (Jin et al., 2023; Liakopoulos et al., 2017; Sroithongkham et al., 2024). Molecular epidemiological studies have documented identical or closely related ESBL-producing strains in dogs and their owners, confirming zoonotic transmission (Jin et al., 2023; Liakopoulos et al., 2017).

Veterinary healthcare workers face occupational exposure to antimicrobial-resistant bacteria through direct contact with infected animals, contaminated environments, and aerosols generated during procedures (Jin et al., 2023).

Implementation of antimicrobial stewardship programs in human hospitals and veterinary practices is one of the one health strategies to optimize antimicrobial use and minimize unnecessary selection pressure (Weese et al., 2019; WHO, 2019).

The One Health perspective emphasizes that combating antimicrobial resistance in companion animal pyometra is not solely about improving individual patient outcomes but represents a responsibility to protect the efficacy of antimicrobials for current and future generations across all species (Velazquez-Meza et al., 2022; WOA, 2024).

AMS represents a systematic, coordinated approach to optimizing antimicrobial use with the overarching goals of improving patient outcomes, minimizing AMR development, reducing adverse effects, and preserving the effectiveness and availability of antimicrobials for current and future generations (AVMA, 2023; Frey et al., 2022; Guardabassi & Prescott, 2015). This proactive strategy requires commitment from all members of the veterinary team, working collaboratively to implement evidence-based antimicrobial use policies (AVMA, 2023; Weese, 2018).

The American Veterinary Medical Association (AVMA) has established principles to guide development and implementation of antimicrobial stewardship programs in veterinary practice (AVMA, 2023; Frey et al., 2022).

Practices should identify high-priority conditions commonly treated with antimicrobials on which to focus stewardship efforts, such as pyometra, urinary tract infections, and skin infections in companion animal practice, and designate one or more individuals to lead the stewardship program and provide accountability (AVMA, 2023).

Prevention represents the most effective antimicrobial stewardship strategy, as diseases that do not occur do not require antimicrobial treatment, and veterinary teams should identify barriers to disease prevention and work with clients to adopt preventive and management strategies that minimize antimicrobial need (AVMA, 2023; Frey et al., 2022). These strategies include appropriate animal husbandry and hygiene, biosecurity and infection control measures, optimal nutrition, comprehensive vaccination programs, routine health monitoring, and parasite control (AVMA, 2023; Frey et al., 2022).

In the context of pyometra prevention, the most effective strategy is elective OVH in dogs and cats not intended for breeding, eliminating the anatomical and hormonal factors that predispose to infection development, and veterinary nurses play crucial roles in client education about preventive care, explaining the rationale for preventive measures and helping overcome barriers to implementation, such as cost concerns or misconceptions about elective surgery (Davidson et al., 2016; Frey et al., 2022; Hagman, 2022).

For pyometra management specifically, judicious antibiotic use involves obtaining uterine samples for culture during OVH, initiating empirical antimicrobial therapy perioperatively based on local resistance patterns, and adjusting therapy based on culture results if treatment extends beyond the perioperative period or if medical management is pursued (Egenvall et al., 2024; Silva et al., 2021).

Antimicrobial stewardship requires ongoing education for all team members to keep current on strategies for disease prevention, antimicrobial alternatives, and evidence-based antimicrobial selection and use, and practices should make resources available and encourage development of expertise in antimicrobial stewardship, critically appraise existing clinical guidelines, and provide client education on stewardship principles including conditions when antimicrobials are not needed (AVMA, 2023; Frey et al., 2022).

Prudent antimicrobial prescribing represents the practical application of antimicrobial stewardship principles at the individual patient level, requiring careful assessment of whether antimicrobial therapy is indicated, selection of the most appropriate agent when treatment is necessary, and optimization of dosing, route, and duration to maximize efficacy while minimizing resistance development and adverse effects (De Briyne et al., 2013; Weese et al., 2015).

The first and most critical decision point in prudent prescribing is determining whether antimicrobial therapy is actually indicated. Antimicrobials should only be used when there is reasonable evidence of bacterial infection that will benefit from treatment (De Briyne et al., 2013; Weese et al., 2015).

For pyometra, antimicrobial therapy is clearly indicated given the severity of uterine bacterial infection and systemic illness, but culture and susceptibility testing should still be performed to enable optimization of antimicrobial selection (Hagman, 2022; Silva et al., 2021).

To guide responsible antimicrobial use and maintain the efficacy of antibiotics, major global health organizations including the WHO, World Organization for Animal Health (WOAH, formerly OIE), and European Medicines Agency (EMA) have developed classification systems that categorize antimicrobials according to their importance in human and veterinary medicine (Collignon et al., 2016; Guardabassi et al., 2023; WHO, 2024). Recognizing that antimicrobials serve essential roles in maintaining animal health and welfare, WOAH developed a parallel classification system focused on veterinary medicine importance (Guardabassi et al., 2023; WOAH, 2021). The WOAH list uses two criteria:

- Criterion 1: The majority of veterinary experts (>50%) confirm the importance of this class of antimicrobials for the treatment of diseases in animals (Guardabassi et al., 2023); Criterion 2: The antimicrobial class is essential for treating specific serious infections in animals and lacks sufficient therapeutic alternatives (Guardabassi et al., 2023).

Based on these criteria, antimicrobials are classified as (Guardabassi et al., 2023; WOAH, 2021):

- Veterinary Critically Important Antimicrobials (VCIA): Classes fulfilling both criteria 1 and 2, including tetracyclines, penicillins, aminopenicillins, cephalosporins, macrolides, fluoroquinolones, aminoglycosides, and trimethoprim-sulfonamide combinations (Guardabassi et al., 2023); Veterinary Highly Important Antimicrobials (VHIA): Classes fulfilling one criterion, including first- and second-generation cephalosporins, lincosamides, and polymyxins (Guardabassi et al., 2023); Veterinary Important Antimicrobials (VIA): Classes fulfilling neither criterion (Guardabassi et al., 2023).

1.5 MANAGEMENT OF PATIENTS WITH PYOMETRA

The management of patients with pyometra begins at admission and extends through surgical intervention, anesthesia, postoperative monitoring, and discharge planning. This comprehensive approach requires coordinated multidisciplinary teamwork to optimize outcomes and reduce complications (Hagman, 2022; Jitpean et al., 2014; Lima et al., 2022).

Upon admission, the initial priority is the rapid assessment and stabilization of the patient. Vital signs, including heart rate, respiratory rate, body temperature, capillary refill time, mucous membrane color, and systolic blood pressure, should be recorded immediately. A thorough physical examination helps identify signs of systemic illness such as lethargy, dehydration, abdominal distention, vaginal discharge, and fever or hypothermia (Jitpean et al., 2014; Lima et al., 2022). Laboratory evaluation is essential and should include a complete blood count, serum biochemistry profile with attention to renal and hepatic function markers, electrolytes, glucose, and plasma lactate concentration. Abdominal imaging via ultrasound or radiography confirms the diagnosis and may reveal complications such as uterine rupture or peritonitis (Hagman, 2022; VCA Hospitals, 2024).

Intravenous access should be established promptly using an appropriately sized catheter in the cephalic or saphenous vein. Isotonic crystalloid fluid therapy is initiated immediately to correct hypovolemia, support tissue perfusion, and address electrolyte imbalances (Lima et al., 2022). Patients presenting with systolic blood pressure below 90mmHg should receive fluid challenge protocols, and if hypotension persists despite volume replacement, early administration of vasopressors such as norepinephrine may be indicated (Lima et al., 2022).

Empiric broad-spectrum intravenous antibiotic therapy is initiated as soon as possible in patients with moderate to severe systemic illness, particularly those with suspected sepsis or peritonitis. Ampicillin or amoxicillin are first-line agents, targeting the most common pathogens, including *E. coli* (Hagman, 2022; Turkki et al., 2023). The timing of antibiotic administration is guided by clinical assessment of the patient's general demeanor, with moderately to severely depressed animals warranting immediate antimicrobial therapy, while stable patients with mild or no depression may not require preoperative antibiotics (Turkki et al., 2023). Pain management is an integral component of initial care, and opioids are administered to provide analgesia and improve patient comfort (Lima et al., 2022).

Once the patient is hemodynamically stable, defined as achieving normotensive blood pressure, adequate urine output, normalized heart rate, and improved mentation, surgical intervention can proceed (Hagman, 2022; Lima et al., 2022). The decision to advance to surgery must balance the need for adequate stabilization against the urgency of removing the infected uterus. Delaying surgery unnecessarily increases the risk of uterine rupture, peritonitis, and septic shock, while premature surgery in an unstable patient elevates anesthetic and surgical risks (Hagman, 2022; Jitpean et al., 2014). Emergency surgery is indicated immediately if there are signs of uterine rupture, uncontrollable sepsis, or rapid clinical deterioration despite aggressive medical management (Hagman, 2022).

Anesthetic management of pyometra patients requires careful consideration of the physiological derangements associated with sepsis and systemic inflammation. Preoperative assessment must evaluate cardiovascular stability, renal and hepatic function, acid-base and electrolyte status, coagulation parameters, and body temperature (Lima et al., 2022; Hagman, 2022). Anesthesia may be maintained with inhalational agents such as isoflurane (Lima et al., 2022).

Intraoperative monitoring is essential and should include continuous electrocardiography, blood pressure measurement, pulse oximetry, capnography, and body temperature monitoring (Lima et al., 2022; Hagman, 2022). Patients are at increased risk of hypotension, arrhythmias, hypothermia, and poor tissue perfusion due to sepsis-induced vasodilation, myocardial depression, and endotoxemia (Lima et al., 2022; Jitpean et al., 2014). In case hypotension persists, despite anesthetic depth adjustment, intervention with additional intravenous fluid boluses or vasopressor administration (Lima et al., 2022).

During OVH, the enlarged, friable, pus-filled uterus must be handled with extreme care to prevent rupture and spillage of infected contents into the abdominal cavity, which would result in life-threatening peritonitis (Hagman, 2022; Turkki et al., 2023). Strict aseptic technique should be maintained throughout the procedure. If inadvertent contamination occurs, copious abdominal lavage with warm sterile saline must be performed immediately, and bacterial culture of peritoneal fluid should be obtained (Turkki et al., 2023). Common intraoperative complications include hemorrhage from ovarian pedicles or uterine vessels, accidental ligation or trauma to the ureters, and anesthetic instability (Jitpean et al., 2014; Hagman, 2022).

Postoperative care begins in the recovery period and continues until the patient is stable for discharge. Intravenous fluid therapy and antimicrobial administration are continued for at least 24 to 48 hours postoperatively in cases that received preoperative antibiotics, with transition to oral antibiotics for a total duration of five to 14 days depending on clinical response and severity of the disease (Hagman, 2022; Turkki et al., 2023). However, recent evidence suggests that antibiotic therapy may be discontinued immediately postoperatively in patients without severe systemic illness, peritonitis, or complications, thereby reducing unnecessary antimicrobial exposure and selection pressure for resistance (Hagman, 2022; Turkki et al., 2023). Analgesia is maintained with opioids and nonsteroidal anti-inflammatory drugs if renal function is adequate. Body temperature, hydration status, appetite, urination, defecation, and surgical site appearance are monitored closely (Hagman, 2022; Jitpean et al., 2014).

Complications that may develop postoperatively include surgical site infections, which occur in approximately 7-11% of the cases not receiving perioperative antibiotics, peritonitis, uterine stump abscessation, wound dehiscence, hemorrhage, and septic shock (Turkki et al., 2023; Jitpean et al., 2014). Surgical site infections typically manifest between four and seven days postoperatively with purulent discharge, wound swelling, erythema, pain, and dehiscence (Turkki et al., 2023). Peritonitis may present with persistent fever, abdominal pain, anorexia, vomiting, and elevated acute phase proteins (Jitpean et al., 2014). Leucopenia is a particularly concerning laboratory finding and is associated with an eighteen-fold increased risk of peritonitis and more than three-fold increased risk of prolonged hospitalization (Jitpean et al., 2014).

Prognosis following OVH is generally favorable, with mortality rates ranging from 1-4% in uncomplicated cases (Hagman, 2022; Jitpean et al., 2014; Turkki et al., 2023). Factors associated with poorer outcomes include severe depression at presentation, leucopenia, peritonitis, uterine rupture, and the presence of multiple organ dysfunctions (Jitpean et al., 2014; Lima et al., 2022). Most patients can be discharged within one to three days postoperatively once they are eating, drinking, urinating normally, afebrile, and demonstrate appropriate wound healing (Hagman, 2022).

Discharge instructions should include guidance on wound care, activity restriction, continuation of oral medications, and monitoring for signs of complications such as lethargy, anorexia, vomiting, abdominal distention, wound discharge, or pyrexia. Owners should be advised to return immediately if any concerning signs develop. A recheck examination is typically scheduled for 7 to 14 days postoperatively to assess healing and remove skin sutures if non-absorbable material was used (Hagman, 2022).

2. Research

2.1 Objectives

The primary objective of the study was to characterize the diversity and prevalence of bacterial species associated with pyometra and to compare their concordance between two anatomical sites, the vagina and the uterus, at a single point in time. Additional objectives were to: (i) describe the epidemiological profile of pyometra cases (species, age, breed and pyometra type), (ii) assess the antimicrobial susceptibility patterns of bacterial isolates using AST, and (iii) determine the prevalence of multidrug-resistant (MDR) isolates, defined as acquired resistance to at least three antimicrobial classes, from vaginal and uterine samples of bitches and queens with pyometra.

2.2 Study design

This study was designed as a prospective longitudinal cohort study, conducted in the small animal surgery service of Hospital Veterinário Escolar da Faculdade de Medicina Veterinária da Universidade Lusófona (FMV-ULHT), located in Lisbon, Portugal. The study forms part of the requirements for the master's degree in veterinary nursing of Companion Animals. Clinical data were collected between September 2024 and December 2025. The study was observational and descriptive–analytical: the investigator did not modify clinical routines or treatment decisions to avoid influencing the occurrence or outcomes of pyometra cases and associated postoperative complications. During the study period, all intact female dogs and cats presenting with clinical suspicion of pyometra and scheduled for OVH were screened for eligibility. From these, 53 cases fulfilled the inclusion criteria and were enrolled, yielding a final sample of 39 surgically confirmed pyometra cases. Each enrolled patient was therefore followed individually from admission, through hospitalization, to discharge.

2.3 Material and methods

2.3.1 Study population and sampling

During the study period, a total of 53 patients presented with clinical suspicion of pyometra. After applying the inclusion and exclusion criteria, 39 cases (27 bitches, 12 queens) with surgically confirmed pyometra and complete paired vaginal and uterine sampling were included in the final analysis.

2.3.2 Inclusion and exclusion criteria

Inclusion criteria comprised intact female dogs and cats with a confirmed diagnosis of pyometra based on clinical signs, laboratory findings and abdominal ultrasonography, that were treated by OVH, with intraoperative collection of both vaginal and uterine swab samples and complete aerobic microbiological processing of these samples, including AST, and for which full clinical records and perioperative follow-up information were available.

Exclusion criteria included animals with suspected pyometra that did not undergo surgery at the hospital, cases in which collection of one or both swabs was not possible or was omitted, culture-invalid or contaminated microbiological samples, and cases with incomplete or missing clinical documentation and data.

2.3.3 Data collection

For each included case, epidemiological and clinical variables were retrieved from the medical records using a standardized data collection form in an Excel spreadsheet. Recorded variables included species, age, breed, pyometra type (open- or closed-cervix), aerobic growth from the uterus and its type (monomicrobial or polymicrobial), aerobic growth from the vagina and its type (monomicrobial or polymicrobial), and antimicrobial susceptibility to the tested antibiotics (cefovecin, ampicillin, amoxicillin, cephalixin, cefotaxime, ceftazidime, gentamicin, levofloxacin, doxycycline, minocycline, trimethoprim, enrofloxacin, fusidic acid, amikacin).

2.3.4 Microbiological procedures

Vaginal and uterine swab samples were obtained aseptically using sterile Amies transport swabs (ref. NM7300287, Deltalab).

Vaginal samples were collected from the cranial vaginal mucosa using a sterile swab and gloves. Whenever possible, sampling was performed at the time of initial diagnosis, before the administration of perioperative antibiotics. When preoperative collection was not possible, the vaginal swab was obtained immediately after induction of anesthesia.

Uterine samples were collected intraoperatively after surgical exteriorization of the uterus. The serosal surface was gently wiped with sterile gauze, and a small hysterotomy was made in a mid-horn area distant from the cervix and ovaries. A sterile swab was inserted into the uterine lumen and rotated against the endometrium to obtain purulent material, avoiding contact with the external uterine surface or surrounding tissues.

Immediately after collection, each swab was placed into its individual transport tube, labelled with the patient identification, sampling site (vagina or uterus) and date. Samples were kept refrigerated at 4–8 °C and transported to the Microbiology Laboratory of Universidade Lusófona for immediate processing and culture. Antimicrobial susceptibility testing (AST) was also performed at this facility, and results were made available through their online platform for subsequent data extraction and analysis.

Microbiological processing was conducted at the Microbiology Laboratory of Universidade Lusófona using selective and differential culture media, including 5% sheep blood agar (Biogerm, SA®), MacConkey agar (Biokar®), Slanetz-Bartley agar (Biokar®) and Brilliance™ MRSA 2 agar (Oxoid). Inoculated plates were incubated aerobically at 37 °C for 24 to 48 hours. Briefly, swab samples were streaked onto the surface of selective and non-selective media to promote growth of Gram-positive cocci, Gram-negative bacilli and potential enterococci or staphylococci. Plates were examined after 24 and 48 hours of incubation for colony morphology, haemolysis patterns and pigment production. Presumptive identification was based on colony characteristics, Gram staining and initial biochemical tests.

Presumptive bacterial colonies underwent DNA extraction followed by PCR amplification targeting the bacterial 16S rRNA gene, with subsequent Sanger sequencing for species-level identification. Sequencing reads were analyzed using [software utilizado, e.g. BLAST or BioEdit] and aligned against reference databases such as NCBI GenBank or the EZBioCloud 16S rRNA database. Identities with sequence similarity ≥99% and good coverage were accepted as definitive species identifications.

Growth was classified as monomicrobial when a single bacterial species was isolated from the sample, and polymicrobial when two or more distinct species were recovered, regardless of colony predominance.

AST was performed by the disk diffusion method (ref), with interpretation of results according to the Clinical and Laboratory Standards Institute (CLSI) veterinary breakpoints (CLSI VET08, 2022). Briefly, isolates were subcultured onto Mueller-Hinton agar to achieve a 0.5 McFarland turbidity standard suspension. Sterile cotton swabs were used to spread the inoculum evenly across the plate surface, antibiotic disks were applied within 15 minutes, and plates were incubated at 35–37 °C in ambient air for 16–18 hours (or 24 hours for fastidious organisms). Zone diameters were measured to the nearest millimeter and interpreted as susceptible (S), intermediate (I) or resistant (R) using CLSI VET08 breakpoints. Quality control was performed using reference strains [e.g. *E. coli* ATCC 25922, *S. aureus* ATCC 25923].

The following antimicrobials were tested: cefovecin, ampicillin, amoxicillin, cephalixin, cefotaxime, ceftazidime, gentamicin, levofloxacin, doxycycline, minocycline, trimethoprim, enrofloxacin, fusidic acid and amikacin.

Susceptibility categories were assigned according to CLSI VET08 zone diameter interpretive criteria: S-indicates that the isolate is likely to respond to the antimicrobial at standard doses, I-suggests that the isolate may respond at higher doses or with favourable pharmacokinetics, R- indicates that the isolate is not inhibited by achievable concentrations of the antimicrobial. For antimicrobials lacking veterinary breakpoints, human CLSI M100 standards were applied with caution.

An isolate was classified as multidrug-resistant (MDR) if it exhibited resistance to ≥ 3 antimicrobial classes from the tested panel, following the harmonized definition proposed by Magiorakos et al. (2012).

2.3.5 Data Analysis

Data were entered and cleaned in Microsoft Excel 365 (Microsoft Corporation, Redmond, WA, USA) and analysed using Jamovi version 2.7.18 (The Jamovi Project, Sydney, Australia)

Description of variables:

Species (dog, cat), age, breed (purebred, mixed), pyometra type (open-cervix, closed-cervix), comorbidities (present, absent), aerobic growth from uterus (no growth, monomicrobial, polymicrobial), aerobic growth from vagina (no growth, monomicrobial, polymicrobial), bacterial concordance between sites (concordant, discordant), susceptibility to each tested antibiotic (S,I,R), MDR (yes, no), defined as resistance to ≥ 3 antimicrobial classes.

Categorical variables were summarised as counts and percentages. Age was described using medians and interquartile ranges due to non-normal distribution. Associations between epidemiological factors and microbiological outcomes (e.g. polymicrobial growth, MDR, concordance) were assessed using chi-square or Fisher's exact tests, as appropriate. Statistical significance was set at $p < 0.05$ (two-tailed).

2.3.6 Ethical considerations

The study was approved by the institutional ethics committee (approval no. 24/2024), ensuring compliance with ethical standards for research involving animals. All data collected were anonymized and confidentiality was strictly maintained throughout the study period, in accordance with institutional and regulatory guidelines.

2.4 RESULTS

2.4.1 Sample Characterization

Of the 39 females included in the study, 27 (69.2%) were dogs and 12 (30.8%) were cats (Figure 4). Among bitches, 17 (60.7%) were mixed-breed and 10 (37.0%) were purebred.

Six canine breeds were represented (Figure 5), with Poodles (n = 3; 10.7%) and Pinschers (n = 2; 7.2%) being the most frequent; Labrador Retrievers, Yorkshire Terriers, Chihuahua Terriers, and French Bulldogs were each represented by a single case (3.7% each). Among queens, all animals (n = 12; 100%) were mixed-breed (Figure 6).

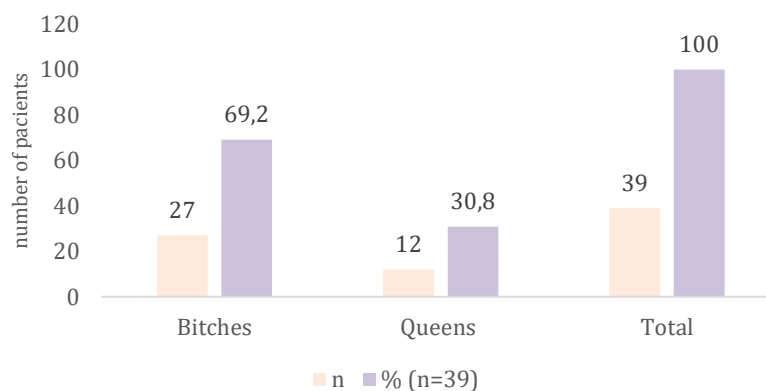


Figure 4- Sample by species

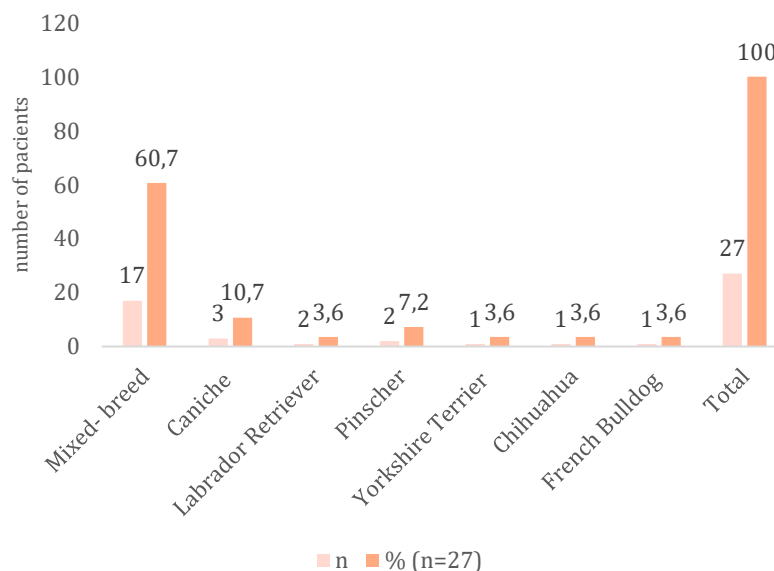


Figure 5- Sample by canine breeds

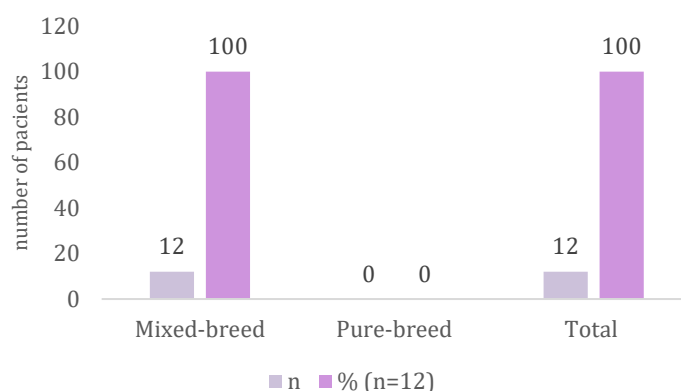


Figure 6- Sample by feline breeds

The age at diagnosis ranged from 2 to 17 years. The mean $\pm$ standard deviation age was 9.3 ± 1.2 years for bitches and 7.1 ± 2.6 years for queens.

At diagnosis, bitches (n = 27; 69.2%) were evenly distributed between open pyometra (n = 14; 50%) and closed pyometra (n = 14; 50%) (Figure 7). In queens (n = 12; 30.8%), open pyometra was more frequent (n = 9; 75.0%) than closed pyometra (n = 3; 25.0%) (Figure 8).

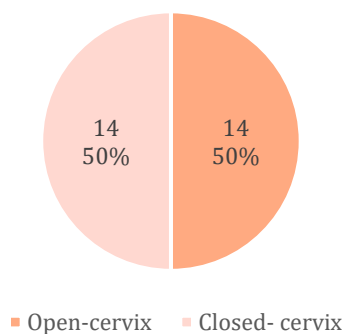


Figure 7- Distribution of bitches according to the type of pyometra

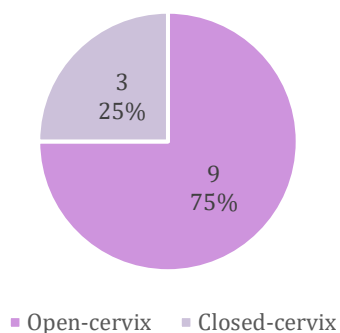


Figure 8- Distribution of queens according to the type of pyometra

Among mixed-breed bitches, 10 cases (58.8%) presented open pyometra and 7 (41.2%) closed pyometra, while the distribution of pyometra type among purebred bitches is shown in (figure 9).

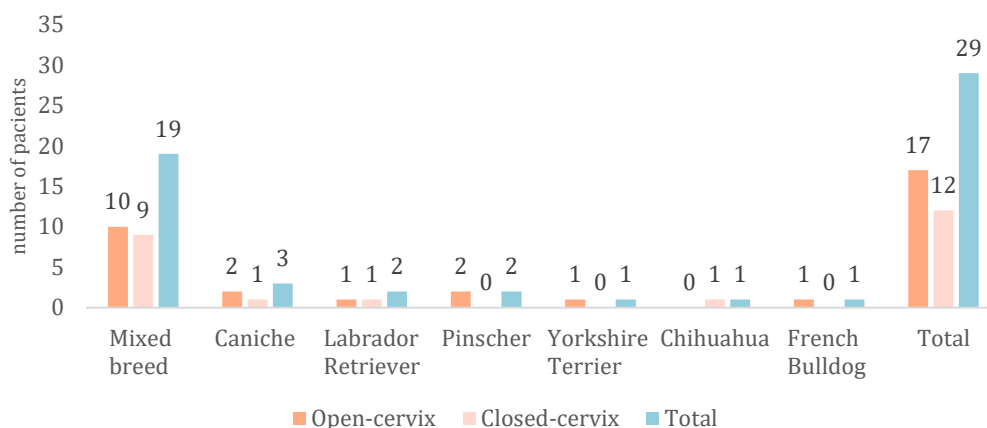


Figure 9- Distribution of canine breeds according to the type of pyometra

2.4.2 Microbiological results

In bitches (n = 27), uterine cultures were predominantly monomicrobial (n = 27; 96.4%), with one case without detectable growth (3.6%). Vaginal samples from bitches yielded monomicrobial growth in 23 cases (82.1%) and polymicrobial growth in 5 cases (17.9%), with no cultures without growth.

In queens (n = 12), uterine cultures in pure culture were observed in 9 cases (81.8%), whereas three queens (18.2%) showed no growth. Vaginal samples from queens showed monomicrobial growth in all cases (n = 11; 100%), with neither polymicrobial cultures nor absence of growth.

In uterine samples from bitches (n = 28), *E. coli* was the most frequently isolated agent (n = 22; 78.6%), occurring in equal numbers in open pyometra (n = 11; 39.3% of open cases) and closed pyometra (n = 11; 39.3% of closed cases), with no statistically significant difference between forms (Fisher's exact test, p = 0.481) (Figure 8). *Haemolytic E. coli* (n = 1; 3.6%), *Enterococcus spp.* (n = 1; 3.6%) and no growth (n = 1; 3.6%) were identified only in open pyometra. *Staphylococcus spp.* (n = 2; 7.1%) and *E. coli ESBL* (n = 1; 3.6%) were detected exclusively in closed pyometra (Figure 10).

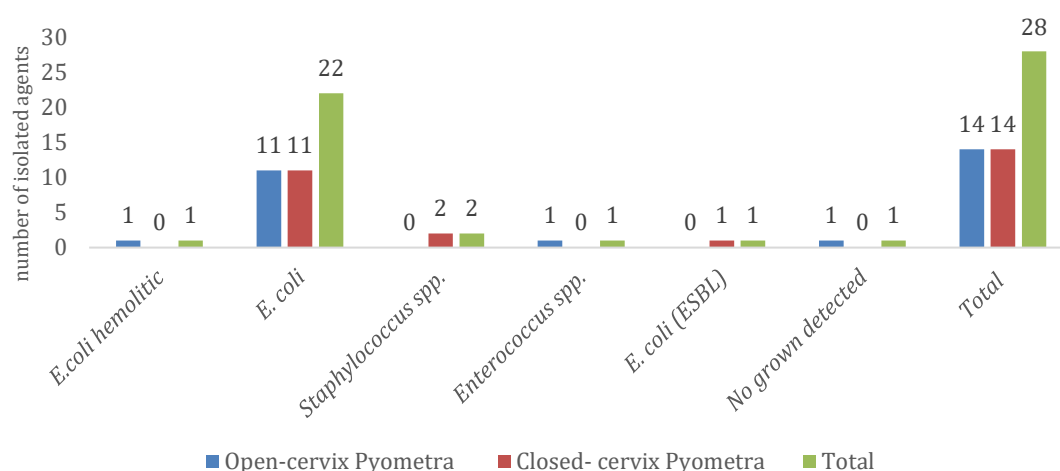


Figure 10- Distribution on bacterial agents isolated from the uterine of the bitches in open- and close cervix pyometra

In vaginal samples from bitches, *E. coli* predominated (n = 19; 67.9%), with a similar distribution between open (n = 9) and closed pyometra (n = 10; Fisher's exact test, p = 1.000) (Figure 9). *Staphylococcus spp.* (n = 3; 10.7%) and *Enterococcus spp.* (n = 3; 10.7%) were the next most frequent agents, also without significant association with pyometra type. *Proteus spp.* (n = 1; 3.6%) occurred only in open pyometra, whereas methicillin-resistant *Staphylococcus pseudintermedius* (MRSP; n = 2; 7.1%) was identified in both open and closed cases (Figure 11).

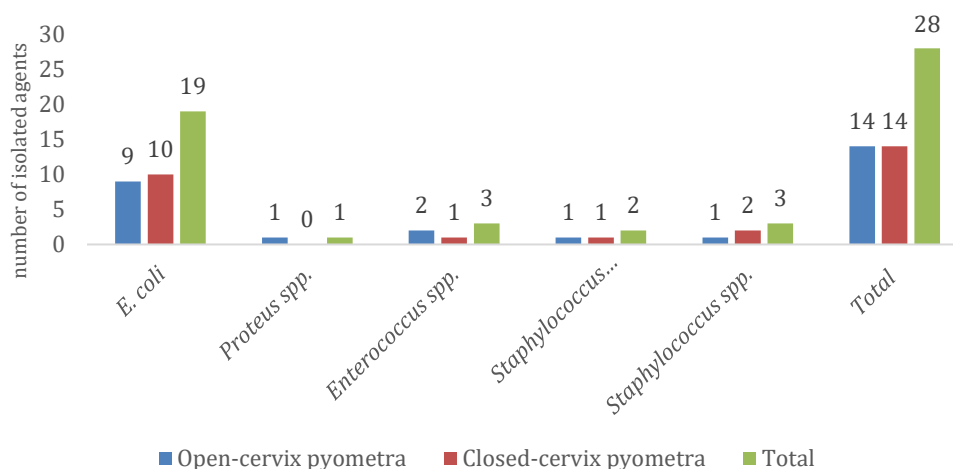


Figure 11- Distribution on bacterial agents isolated from the vaginal of the bitches in open- and close-cervix pyometra

In uterine samples from queens (n = 11), *E. coli* was again the most frequent agent (n = 3; 27.3%), identified only in open pyometra (Figure 12). Other agents—*Staphylococcus* spp., *Enterococcus* spp., *Streptococcus* spp. and *E. coli* ESBL (each n = 1; 9.1%)—also occurred exclusively in open pyometra, whereas no growth (n = 3; 27.3%) was observed in both forms (n = 2 open; n = 1 closed). No statistically significant differences were found in uterine agent distribution by pyometra type (Fisher's exact test, p = 0.618).

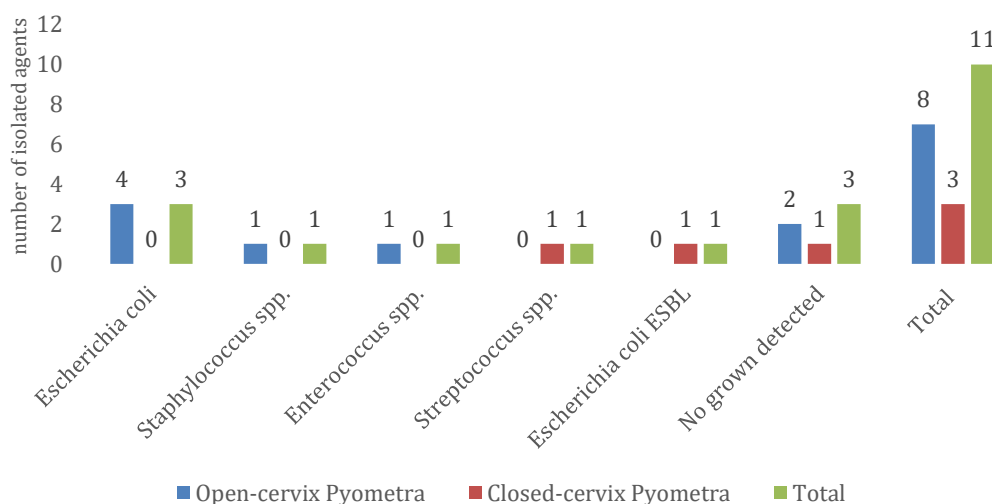


Figure 12- Distribution on bacterial agents isolated from the uterine of the queens in open- and close- cervix pyometra

In vaginal samples from queens, *Staphylococcus* spp. (n = 5; 45.5%) and *E. coli* (n = 4; 36.4%) were the most frequent agents, mainly in open pyometra (n = 4 and n = 4, respectively) (Figure 13). *Streptococcus* spp. and *E. coli* ESBL (each n = 1; 9.1%) were detected only in closed pyometra. A non-significant trend towards greater bacterial diversity in open pyometra was observed (Fisher's exact test, p = 0.079).

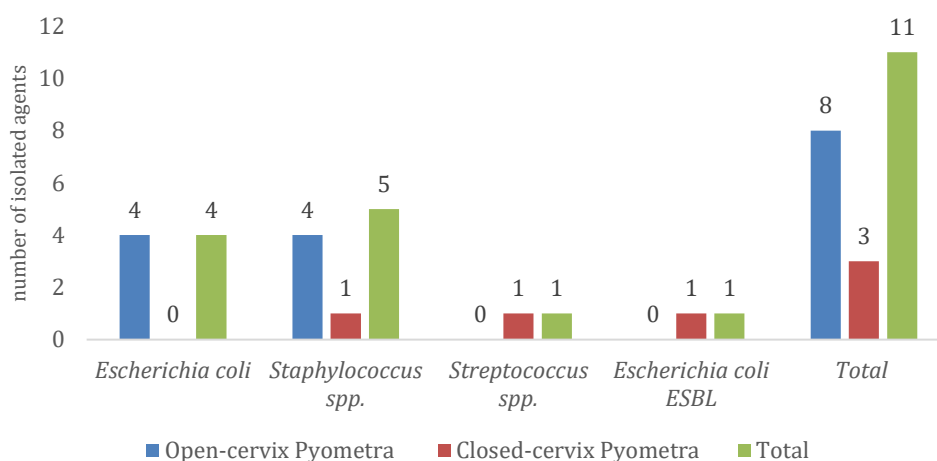


Figure 13-Distribution on bacterial agents isolated from the vaginal of the queens in open- close-cervix pyometra

In canine cases with paired samples (n = 34), agreement between uterine and vaginal bacterial agents was observed in 18 cases (52.9%), whereas 16 cases (47.1%) showed discordant isolates between sites. In queens (n = 11), agreement between uterine and vaginal isolates occurred in 7 cases (63.6%), and 4 cases (36.4%) were discordant.

Agreement between uterine and vaginal isolates in bitches did not differ significantly between open pyometra (n = 4; 50.0%) and closed pyometra (n = 1; 33.3%) among cases with multiple uterine sampling sites ($\chi^2 = 0.36$, 1 df, p = 0.548).

Considering all aerobic bacterial isolates from bitches with open-cervix pyometra, susceptibility remained high for most antimicrobials tested (Figure 14). Overall, $\geq 90\%$ of isolates were categorised as susceptible to cefovecin (93.8%, 30/32), amoxicillin–clavulanic acid (78.1%, 25/32), cephalothin (75.0%, 24/32), cefotaxime (93.8%, 30/32), ceftazidime (93.8%, 30/32), gentamicin (93.8%, 30/32), levofloxacin (87.5%, 28/32), minocycline (84.4%, 27/32), trimethoprim/sulfamethoxazole (90.6%, 29/32) and enrofloxacin (81.3%, 26/32), with low proportions of intermediate (0–15.6%) and resistant (0–28.1%) isolates. Doxycycline showed slightly lower susceptibility (71.9%, 23/32), with 6.3% (2/32) intermediate and 21.9% (7/32) resistant isolates. Ampicillin exhibited the least favourable profile, with only 56.3% (18/32) susceptible isolates and 28.1% (9/32) resistant, indicating a more pronounced resistance to this beta-lactam in open-cervix cases.

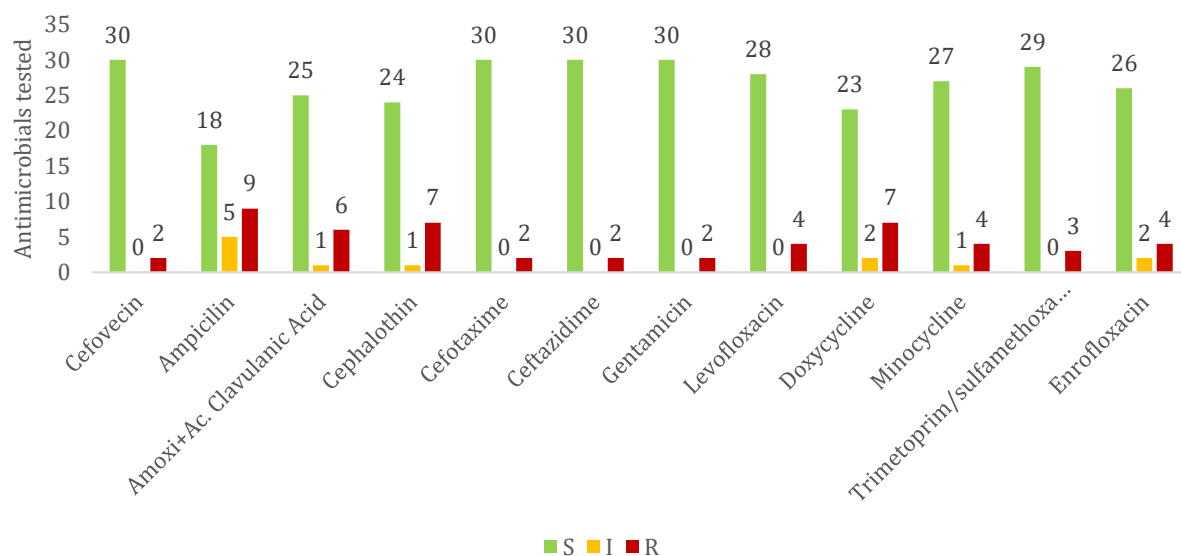


Figure 14- Antimicrobial susceptibility of bacterial agents isolated in open-cervix in bitches

According to the European Medicines Agency (EMA) categorisation, most of the antimicrobials showing high proportions of susceptible isolates in this group (e.g. amoxicillin–clavulanic acid, first-generation cephalosporins, doxycycline, trimethoprim/sulfamethoxazole) belong to category D (‘Prudence’), whereas fluoroquinolones such as enrofloxacin and levofloxacin are classified as category B (‘Restrict’), underscoring the need to reserve them for cases where first-line options are unsuitable.

In bitches with closed-cervix pyometra, a similar pattern was observed (Figure 15). Cefovecin (87.1%, 27/31), amoxicillin–clavulanate (83.9%, 26/31), cephalothin (90.3%, 28/31), cefotaxime (90.3%, 28/31), ceftazidime (90.3%, 28/31), gentamicin (100%, 31/31) and trimethoprim/sulfamethoxazole (100%, 31/31) showed high proportions of susceptible isolates, with very few intermediate or resistant strains. Levofloxacin (87.1%, 27/31) and minocycline (83.9%, 26/31) also showed good activity. Doxycycline again had slightly lower susceptibility (77.4%, 24/31), mainly due to an increased proportion of intermediate isolates. Ampicillin remained the antimicrobial with the highest frequency of non-susceptible isolates (74.2% susceptible, 6.5% intermediate and 19.4% resistant).

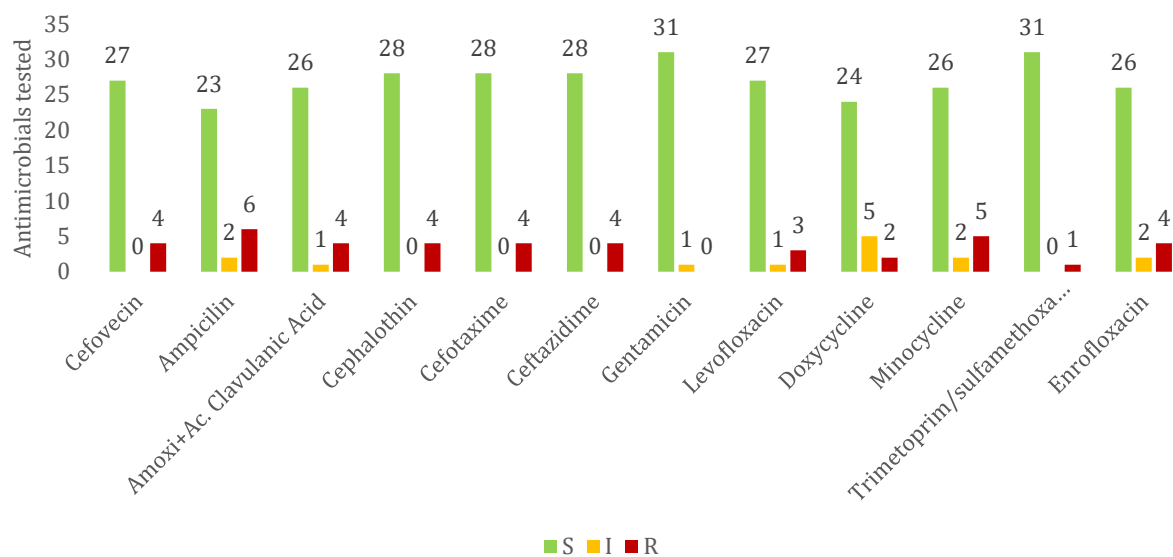


Figure 15-Antimicrobial susceptibility of bacterial agents isolated in closed-cervix in bitches

Among isolates from queens with open-cervix pyometra, the overall susceptibility profile was very favourable (Figure 16). All isolates (100%, 14/14) were susceptible to cefotaxime, ceftazidime, gentamicin, levofloxacin, doxycycline, minocycline and trimethoprim/sulfamethoxazole. For cefovecin, ampicillin, amoxicillin–clavulanate and cephalothin, 92.9% (13/14) of isolates were susceptible and 7.1% (1/14) resistant. Enrofloxacin showed 85.7% (12/14) susceptible, 7.1% (1/14) intermediate and 7.1% (1/14) resistant isolates.

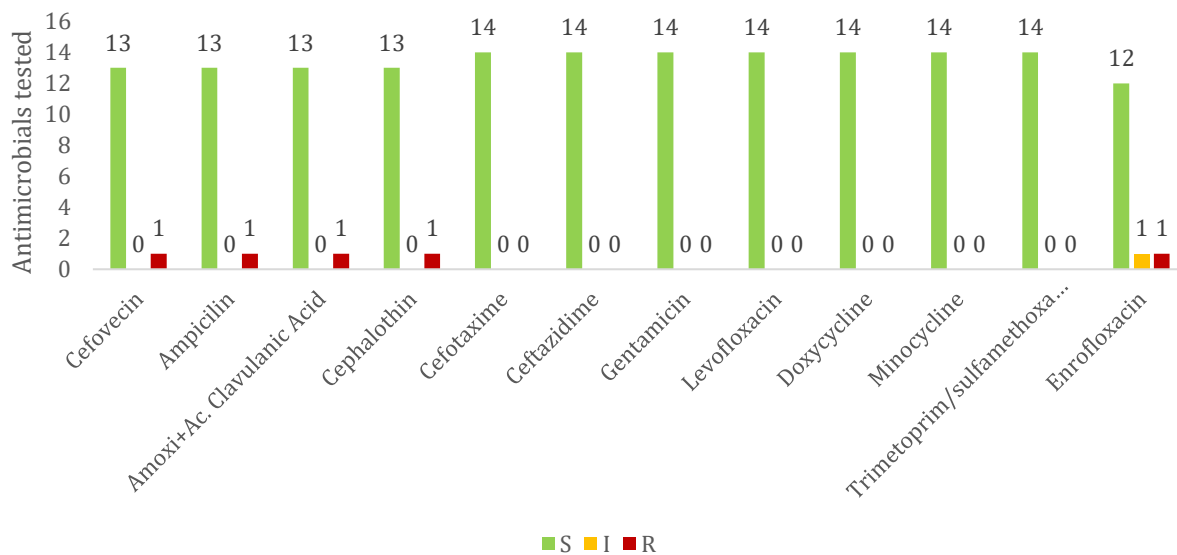


Figure 16- Antimicrobial susceptibility of bacterial agents isolated in open-cervix in queens

In queens with closed cervix pyometra, the number of isolates was low (n = 5 per antimicrobial), and results should be interpreted with caution (Figure 17). For cefovecin, ampicillin, amoxicillin–clavulanate, cephalothin, cefotaxime, ceftazidime and gentamicin, 60.0% (3/5) of isolates were susceptible and 40.0% (2/5) resistant. All isolates (5/5; 100%) were susceptible to levofloxacin, doxycycline, minocycline and trimethoprim/sulfamethoxazole. For enrofloxacin, 60.0% (3/5) of isolates were susceptible and 40.0% (2/5) intermediate, with no resistant strains.

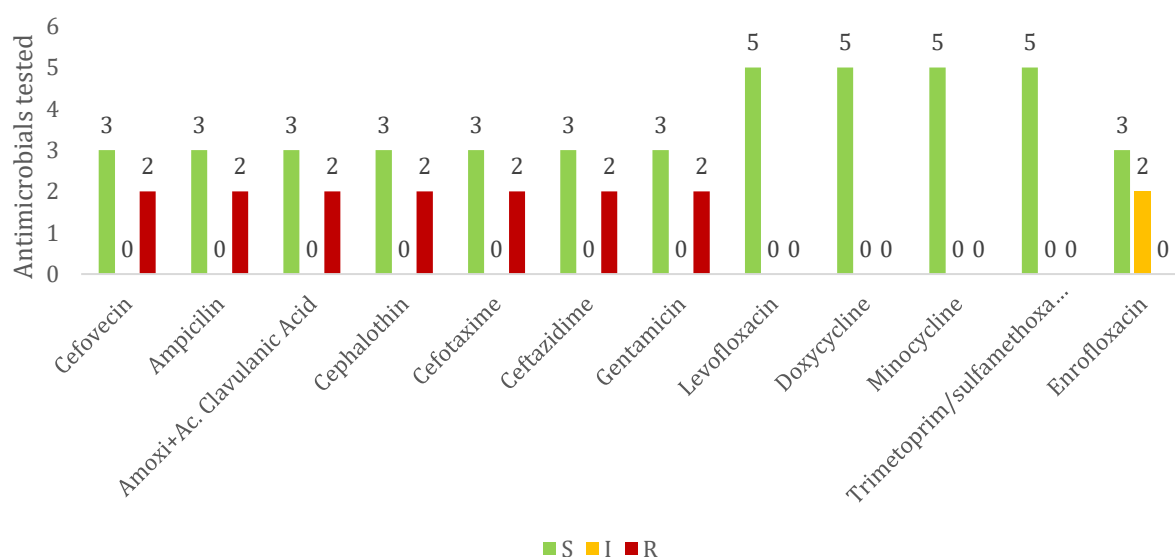


Figure 17-Antimicrobial susceptibility of bacterial agents isolated in closed-cervix in queens

3. DISCUSSION

Pyometra was more frequently diagnosed in bitches (69.2%) than in queens (30.8%), which agrees with previous studies indicating that this condition is more common in dogs than in cats (Hollinshead & Krekeler, 2016). This difference is likely related to species-specific reproductive physiology, particularly the prolonged diestrus phase in bitches that results in repeated and sustained progesterone exposure of the endometrium, promoting cystic endometrial hyperplasia and predisposing the uterus to bacterial colonisation (Hagman, 2023). The age distribution observed in the present study, with mean ages of 9.3 years in bitches and 7.1 years in queens, corroborates the well-established association between pyometra and increasing age, reflecting the cumulative effects of hormonal stimulation on the uterus over multiple oestrous cycles and the progressive development of endometrial pathology (Hagman, 2023). The predominance of mixed-breed animals, particularly among queens, probably mirrors the caseload of the hospital rather than indicating a lack of breed predisposition; indeed, several canine breeds have been reported at higher risk for pyometra in population-based studies (Hagman, 2023).

In the present series, open- and closed-cervix pyometra occurred at similar frequencies in bitches, whereas queens were more frequently affected by the open-cervix form, a pattern in line with clinical descriptions of feline pyometra (Hollinshead & Krekeler, 2016). These interspecies differences may be explained by variations in cervical physiology, hormonal patterns and reproductive mechanisms, particularly the induced ovulation characteristic of queens, which may favour cervical patency and the earlier onset of vulvar discharge, thereby facilitating clinical recognition and influencing case presentation (Hollinshead & Krekeler, 2016). The higher proportion of open-cervix pyometra in queens may thus contribute to earlier diagnosis, whereas closed-cervix disease in dogs can be associated with more severe systemic illness at presentation (Hagman, 2023).

Uterine cultures were predominantly monomicrobial, especially in bitches (96.4%), which is consistent with previous reports describing canine and feline pyometra as usually caused by a single dominant pathogen rather than polymicrobial infection (Hagman, 2023; Silva et al., 2023). Across both species and sampling sites, *Escherichia coli* was the most frequently isolated bacterial species, particularly in uterine samples from bitches (78.6%), reinforcing its central role as the primary etiological agent of pyometra in small animals (Silva et al., 2021; de Campos et al., 2022). This predominance of extraintestinal pathogenic *E. coli* is in agreement with studies demonstrating that pyometra isolates share virulence factor profiles with strains recovered from urinary tract infections and faeces of healthy dogs, supporting a common pathogenesis based on ascending infection from the lower urinary or gastrointestinal tract (Johnson et al., 2009; de Campos et al., 2022). Other bacterial genera, including *Staphylococcus* spp. and *Enterococcus* spp., were identified less frequently and mostly in mixed cultures, suggesting an opportunistic or secondary role that likely reflects alterations in the genital microbiota and the favourable, progesterone-dominated uterine environment rather than a distinct primary infection (Silva et al., 2023).

Agreement between vaginal and uterine isolates was moderate in bitches (52.9%) and slightly higher in queens (63.6%), with approximately half of the cases showing discordant flora between the two sites. Concordance in the range of 50–65% is generally interpreted as partial or moderate and is comparable to the agreement reported in previous microbiological studies of canine pyometra (Silva et al., 2023). These findings indicate that vaginal cultures are not a fully reliable proxy for uterine infection: although they can provide useful preliminary information, they do not consistently reproduce the microbiological profile of the uterus and therefore should not be regarded as a definitive surrogate for uterine sampling when precise pathogen identification is required for targeted antimicrobial therapy (Silva et al., 2023; Hagman, 2023). Nonetheless, vaginal swabs retain clinical utility as an initial, minimally invasive diagnostic tool and as a guide to early empirical antimicrobial treatment, particularly for identifying *E. coli* and screening for multidrug-resistant strains before surgery (Silva et al., 2023).

Antimicrobial susceptibility patterns in this study were generally favourable. Most isolates from both species remained susceptible to cefovecin, amoxicillin–clavulanate, gentamicin and cephalosporins, in agreement with recent data showing good in vitro activity of these agents against *E. coli* and other pathogens isolated from canine pyometra and small-animal infections (Silva et al., 2023; Johnson et al., 2009). These results support the continued use of cefovecin and amoxicillin–clavulanate as reasonable empirical options in many pyometra cases, particularly when culture results are not yet available and systemic infection is suspected. In our isolates, both drugs retained high levels of in vitro activity, which is in line with current recommendations to prioritise older, narrow- or moderate-spectrum agents before resorting to higher-risk classes in veterinary medicine. According to the European Medicines Agency, cefovecin and aminopenicillins combined with beta-lactamase inhibitors are classified in group D (“Prudence”), meaning they should be used thoughtfully but can be considered first-line choices when indicated, provided that dosages, treatment duration and local resistance data are carefully taken into account. By contrast, ampicillin showed the highest proportion of resistant isolates, consistent with previous reports of widespread resistance to narrow-spectrum beta-lactams among canine *E. coli*. This pattern suggests that ampicillin monotherapy should no longer be used as an empirical first-line choice for pyometra, except where recent culture and susceptibility results clearly support its use.

Fluoroquinolones such as enrofloxacin and levofloxacin demonstrated good in vitro efficacy, but they are classified by the European Medicines Agency as group B (“Restrict”), meaning they should be reserved for selected, culture-guided indications when no suitable alternatives from categories C or D exist, owing to their critical importance in human medicine and the need to limit resistance selection (European Medicines Agency, 2019). The prevalence of multidrug-resistant isolates observed in the present work is clinically relevant and comparable to recent veterinary reports documenting increasing multidrug resistance among *E. coli* from canine reproductive and urinary tract infections (Silva et al., 2023; de Campos et al., 2022). This situation reflects growing selective pressure from antimicrobial use and underscores the importance of routine culture and susceptibility testing, targeted rather than empirical long-term therapy, and strict adherence to antimicrobial stewardship principles in small-animal practice (Hagman, 2023).

Interpretation of these findings must consider several limitations. The overall sample size was modest—especially for queens and for closed-cervix cases—reducing statistical power and the ability to detect subtle differences between groups, as highlighted in earlier clinical series of pyometra (Hollinshead & Krekeler, 2016; Hagman, 2023). Microbiological investigation relied on single vaginal and uterine swabs obtained under clinical conditions, without quantitative culture or molecular typing; as a result, contamination, under-representation of minor bacterial populations, and the inability to determine clonal relatedness between isolates cannot be excluded (Silva et al., 2023). Only aerobic bacteria were assessed, and antimicrobial susceptibility testing was performed using disk diffusion with a limited panel of drugs, which may underestimate resistance in fastidious or anaerobic organisms and does not provide minimum inhibitory concentration data (Silva et al., 2023). In addition, in a substantial number of cases, perioperative antimicrobial therapy had already been initiated before collection of the vaginal swab, potentially reducing bacterial recovery and altering the composition of the vaginal microbiota, thereby influencing the apparent concordance with uterine isolates (Silva et al., 2023).

Despite these constraints, the present study contributes novel combined information on vaginal and uterine microbiota and corresponding susceptibility patterns in bitches and queens with pyometra, interpreted within current multidrug-resistance and EMA categorization frameworks. By discussing each main result—species and age distribution, form of disease, microbiological profile, agreement between sampling sites and susceptibility patterns—the study provides clinically useful guidance for empirical antibiotic selection, clarifies the strengths and limitations of vaginal cultures as a surrogate for uterine infection and supports more responsible antimicrobial use in the management of pyometra in small-animal veterinary practice (Hagman, 2023; European Medicines Agency, 2019).

4. CONCLUSION

Taken together, the findings of this study confirm that pyometra remains a common and clinically significant condition in both bitches and queens, with important implications for individual patient management and for antimicrobial use in small-animal practice. The integrated analysis of clinical data, microbiology, and susceptibility patterns shows that, despite some species-related differences, the pathogenesis and bacteriological profile of the disease are largely comparable between dogs and cats. This supports the view that pyometra should be approached as a systemic infectious disease superimposed on a hormonally altered uterus, rather than as a purely surgical problem, and that diagnostic and therapeutic decisions should systematically incorporate microbiological information.

From a practical standpoint, the results highlight several aspects that can directly inform clinical decision-making. The high proportion of monomicrobial infections and the predominance of *Escherichia coli* as the main uterine pathogen support targeted empirical protocols that prioritise coverage of this species, while recognising that other bacteria may play a secondary or opportunistic role. The generally favourable susceptibility to cefovecin and amoxicillin–clavulanate, together with the high levels of resistance to ampicillin and the critical-importance status of fluoroquinolones, provide a clear rationale for choosing first-line agents that balance efficacy with stewardship principles. In parallel, the only moderate agreement between vaginal and uterine isolates indicates that, although vaginal swabs can be useful as an initial, minimally invasive tool—especially when uterine sampling is not immediately feasible—they cannot fully replace direct uterine cultures when precise pathogen identification and optimisation of therapy are required.

The study also has broader implications for antimicrobial stewardship. The identification of a relevant proportion of multidrug-resistant isolates, even in a relatively small cohort, underlines that pyometra can contribute to the overall reservoir of resistant bacteria in companion animals. By documenting resistance patterns in detail and interpreting them within the framework of current EMA categories, this work reinforces the need to reserve critically important antimicrobials for culture-guided situations and to avoid outdated empirical choices such as ampicillin monotherapy. In this way, the study supports a more structured, evidence-based approach to perioperative and postoperative antimicrobial use in pyometra cases, which can be readily implemented in general practice and referral settings.

Several limitations of the present investigation must be recognised. The sample size, particularly for queens and closed-cervix cases, restricts the power to detect subtle differences related to species, cervical status, or other clinical variables. The reliance on single, qualitative swabs and conventional culture methods, with a focus on aerobic bacteria and a finite panel of antimicrobials, limits the ability to fully characterise the complexity of the uterine and vaginal microbiota, to assess anaerobic or fastidious organisms, and to determine minimum inhibitory concentrations. Moreover, the fact that some vaginal samples were collected after the initiation of antimicrobial therapy may have reduced bacterial recovery and influenced the apparent level of agreement between vaginal and uterine isolates. These factors mean that the true diversity of pathogens and the prevalence of resistance may be somewhat underestimated.

Despite these constraints, the study offers several clear strengths and positive contributions. It provides a simultaneous, side-by-side description of canine and feline pyometra within the same clinical environment, integrating clinical presentation, anatomical form of disease, bacteriology and susceptibility data rather than treating these aspects in isolation. It also explicitly links laboratory findings to current antimicrobial-use guidelines, translating microbiological results into concrete recommendations that clinicians can apply when selecting empirical and definitive therapy. Finally, by quantifying the concordance between vaginal and uterine cultures and discussing its practical implications, this work clarifies the role and limitations of less invasive sampling strategies and supports a more judicious, rational use of antimicrobials in the management of pyometra. In combination, these elements mean that, beyond characterising the local caseload, the study provides actionable information that can improve patient outcomes and contribute to more responsible antimicrobial stewardship in small-animal practice.

DISSEMINATION OF RESULTS

The main findings of this dissertation have already been disseminated at a national scientific meeting in the form of conference posters:

Martins, J., Cardoso, D., Dias, B., Pancrácio, D., Campos, S., Martins, L., Belas, A. "Characterization of antimicrobial resistance in pyometra of companion animals in the Lisbon Metropolitan area." Poster presented at the XXI Congresso Internacional Veterinário Montenegro 2025, Santa Maria da Feira, Portugal.

Pancrácio, D., Martins, J., Belas, A., Campos, S., Martins, L., Cardoso, D., Borges, P., Dias, B., Pereira, M. "Comparação entre a flora bacteriana vaginal e uterina nas piometras de animais de companhia." Poster presented at the XXI Congresso Internacional Veterinário Montenegro 2025, Santa Maria da Feira, Portugal. Awarded the Best Scientific Poster in Veterinary Nursing Award.

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