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MICROBIOLOGICAL AND HISTOPATHOLOGICAL INSIGHTS ON CANINE AND FELINE PYOMETRA

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ABSTRACT

Pyometra is a life-threatening condition affecting both bitches and queens, which should be diagnosed and treated early to save the animal's life. This study aimed to identify the recent bacterial isolates associated with pyometra and determine the most effective antibiotic for the treatment according to the sensitivity test. Histopathological examinations have also been performed. The study involved 17 queens and 8 bitches presented to the Teaching Hospital of the Faculty of Veterinary Medicine, Mansoura University, with a mean age of 4.56 years for queens and 4.75 years for bitches. Diagnosis of pyometra depended on history, clinical signs, physical examination, and ultrasonographic imaging. After ovariohysterectomy (OHE), the samples were collected under complete aseptic conditions for bacterial isolation and identification. The study revealed that the most common isolated bacteria from both bitches and queens was *E. coli,* which represents 37.5% and 47%, respectively. Other bacterial species were isolated, such as *Klebsiella*, *Enterobacter*, and *Proteus*. Some samples showed no growth, which may be attributed to the antimicrobial treatment before the collection of samples, the infection with anaerobic bacteria, or misdiagnosis with mucometra or hydrometra, which are usually not associated with infection. The most sensitive antimicrobial drug, doxycycline (DO), emerged as the most effective antimicrobial, with 83.3% of bacteria being sensitive. Some resistance genes for antibiotics have been detected using PCR, such as *blaTEM* and *blaSHV* in E. coli, which were responsible for the resistance to the beta-lactam group, and the *qnrS* gene encodes resistance to the quinolones. Histologically, there was heavy lymphocytic cell infiltration with cystic endometrial glands and endometrial hemorrhage. In conclusion, early detection and careful monitoring of antimicrobial susceptibility are crucial before therapeutic intervention to decrease the chance of antimicrobial resistance.

Key words: Bitches, queens, Doxycycline, Cystic Endometrial Hyperplasia

INTRODUCTION

Pyometra is a common and potentially life-threatening reproductive dis-order in mid- to old-aged bitches and queens. It may affect about 20% of all bitches and 2.2% of all queens. It is characterized by the accumulation of pus inside the uterine lumen, which may cause pathological changes in the endometrium and systemic illness (Jitpean *et al.,* 2014). Pyometra typically develops during the luteal phase of the estrous cycle, as the progesterone concentration is high, making

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the uterus more susceptible to bacterial infection. *Escherichia coli* is the most frequently isolated bacteria from the affected uterus in both species (Wadås *et al.,* 1996). The prevalence of *E. coli*-induced pyometra could be attributed to adherence via the K antigen, which is necessary for colonizing the microorganism during infection with pyometra (Sandholm *et al.,* 1975). The high level of estrogen during estrus enhances the development of progesterone receptors in the endometrium, followed by a high level of progesterone during the luteal phase (Prapaiwan *et al.,* 2017). This leads to an increase in the growth and multiplication of endometrial glands, which in turn causes an increase in endometrial secretion (Rautela and Katiyar, 2019).

Cystic endometrial hyperplasia (CEH) frequently precedes pyometra, creating a pathological state that predisposes the uterus to infection (De Bosschere *et al.,* 2001). Pyometra can be classified as open or closed, depending on the presence or absence of vaginal discharge. Other clinical symptoms may appear, such as nausea, lethargy, and vomiting. Ovario-hysterectomy (OHE) is the treatment of choice, as there's no chance for recurrence and the source of infection is removed (McCobb *et al.,* 2022). However, medical treatment may be a choice using ecbolic drugs together with antimicrobial drugs, based on antimicrobial sensitivity tests (Verstegen *et al.,* 2008).

This study aims to investigate the most prevalent bacterial agents associated with pyometra in both bitches and queens, and to determine the most effective antimicrobial agents for medical management based on sensitivity testing.

MATERIALS AND METHODS

A. Animals:

This study was performed on 25 females, including 17 queens and 8 bitches, diagnosed with pyometra between January 2022 and July 2024. Diagnosis was based on case history, vaginal discharge in case of an opened cervix, and confirmed by ultrasound imaging (CHISON, ECO 1, CHISON Medical Imaging Co., Ltd., Wuxi, China).

All animals were admitted to the Teaching Hospital of the Faculty of Veterinary Medicine, Mansoura University. The study was conducted in accordance with *the Guidelines for the Husbandry and Use of Farm Animals in Research and Teaching (3rd edition*) (http://www.fass.org/) and approved by the Animal Welfare and Ethics Committee of Mansoura University, Mansoura, Egypt.

B. Study design:

The study included the following procedures: bacterial isolation and identification, antimicrobial susceptibility testing, detection of resistance genes via PCR, and histopathological examination.

35. Bacteriological isolation and identification:

Samples were taken from 17 queens and 8 bitches following ovariohysterectomy after being diagnosed with pyometra, which was confirmed by clinical examination and ultrasonography. The samples were taken from the affected uterus under aseptic conditions after surgery. Identification of bacterial isolates was based on colony morphology, Gram staining characteristics, and standard biochemical tests (according to Christopher and Bruno (2003).

Swabs were inoculated onto nutrient agar, eosin methylene blue agar, and S-S agar (all from Oxoid) and incubated at 37°C overnight. The grown colonies were subjected to biochemical tests, including oxidase, catalase, and urease.

On eosin methylene blue agar (EMB), *E. coli* showed a characteristic green sheen, indicating vigorous lactose fermentation and acid production, which precipitates the green metallic pigment. *Enterobacter*

showed good growth of brown, dark-centered colonies, indicating lactose fermentation and acid production, and *Klebsiella* showed good growth of brown, dark-centered, mucoid colonies.

On S-S agar, the *E. coli* colonies appeared pink, while the *Enterobacter* and *Klebsiella* colonies were larger compared to *E. coli*. The *Enterobacter* and *Klebsiella* colonies had a mucoid, pale, opaque cream-to-pink appearance.

B. 2. Antimicrobial resistance:

Phenotypic resistance was determined using Kirby-Bauer's disc diffusion method, following Clinical and Laboratory Standards Institute (CLSI) standards and interpretation criteria (1, 2, 3). (Marques *et al.,* 2018). The antibiotics used in the current study for sensitivity were vancomycin (VA, 10 µg), ampicillin (AM, 10 µg), ciprofloxacin (CIP, 30 µg), sulfamethoxazole/trimethoprim (COT, 25 µg), amoxicillin/clavulanic acid (AMC, 30 µg), ampicillin/sulbactam (A/S, 10/10 µg), gentamicin (CN, 10 µg), cefadroxil (CFR, 30 µg), cefuroxime (CXM, 30 µg), and doxycycline (DO, 30 µg).

The first pure 4-5 colonies were selected out from overnight culture. The colonies are transferred into a sterile capped glass tube that contains a sterile broth mix using a vortex mixer. A spectrophotometer is used to assess turbidity. The absorbance range should match the McFarland standard 0.5 (OD625 nm: 0.08–0.13).

A sterilized cotton swab was Immersed In the broth culture tube. According to the Kirby-Bauer method, the swab was streaked on the surface of the nutrient agar medium in 3 planes: horizontal, vertical, and diagonal (Bauer *et al.,* 1966). Using sterile forceps, the discs were placed one at a time on the agar surface after the inoculum had dried for three to five minutes. They were then gently pressed down to ensure even contact. Discs should not be removed after they contact the agar surface because some of the antibacterial compounds in them disperse nearly instantly. The plates were incubated aerobically for 18-24 hours at 37±1ºC.

A ruler from the back of the plates measured the diameter of the inhibition zone around each disc. To ascertain whether the organism is susceptible (S), intermediate (I), or resistant I to the antimicrobial drugs utilized, the results were compared to a standardized chart provided by Oxoid-England.

B. 3. PCR:

Genomic DNA was extracted according to QIAamp DNA mini kit instructions. Catalogue no. 51304 was used for PCR. The preparation of PCR master mix for PCR according to Emerald Amp GT PCR master mix (Takara) Code No. RR310A kit is shown in Table (1). The primers, time and temperature scheme of every gene were summarized in Tables (2 and 3).

Table 1: Preparation of PCR Master Mix for PCR

Component	Volume/ reaction
EmeraldAmpGT PCR mastermix (2x premix)	12.5 µl
PCR-grade water	5.5 µl
Forward primer (20 pmol)	1 µl
Reverse primer (20 pmol)	1 µl
Template DNA	5 µl
Total	25*µl*

0.5 µg/ml ethidium bromide was added and thoroughly mixed after electrophoresis-grade agarose (1.5 g) was prepared in 100 ml TBE buffer in a sterile flask, heated in the microwave to dissolve all granules with agitation, and allowed to cool at 70°C. With the desired comb in place, the warm agarose was poured straight into the gel casting equipment and allowed to polymerize at room temperature. After removing the comb, TBE buffer was added to the electrophoresis tank. The gel was loaded with 20 µl of each uniplex PCR product

sample, negative control, and positive control. The power supply was 1-5 volts/cm of the tank length. After around half an hour, the run ceased, and the gel was moved to the UV cabinet. The gel was photographed using a gel documentation system, and computer software was used to evaluate the data. E. coli isolates from dogs and cats were tested for the presence of cephalosporins and ß-lactam-resistant genes (*blaTEM, blaSHV*) and for the presence of genes conferring resistance to quinolones (*qnrS, qepA*). Specific primers were used to amplify the operons of the target genes by PCR.

Table 2: The primers used in the PCR

Target gene	Primer sequence (5'-3')	Length of amplified product (bp)	Reference
Pseudomonas *16S rDNA*	GACGGGTGAGTAATGCCTA	618	**Spilker *et al*., 2004**
	CACTGGTGTTCCTTCCTATA		
Klebsiella *gyrA*	CGC GTA CTA TAC GCC ATG AAC GTA	411	**Brisse and Verhoef, 2001**
	ACC GTT GAT CAC TTC GGT CAG G		
E. coli phoA	CGATTCTGGAAATGGCAAAAG	720	**Hu *et al.*,2011**
	CGTGATCAGCGGTGACTATGAC		
qnrS	ACGACATTCGTCAACTGCAA	417	**Robicsek *et al*., 2006**
	TAAATTGGCACCCTGTAGGC		
BlaTEM	ATCAGCAATAAACCAGC	516	**Colom *et al*., 2003**
	CCCCGAAGAACGTTTTC		
BlaSHV	AGGATTGACTGCCTTTTTG	392	
	ATTTGCTGATTTCGCTCG		
qepA	CGTGTTGCTGGAGTTCTTC	403	**Cattoir *et al*., 2008**
	CTGCAGGTACTGCGTCATG		
Proteus *atpD*	GTATCATGAACGTTCTGGGTAC	595	**Bi *et al*., 2013**
	TGAAGTGATACGCTCTTGCAG		
Enterobacter *gyrB*	GGCAAAGCTCAACCCGGAGGTATTCT	414	**Asselin et al., 2016**
	CAAAGAAAGATAATAATTTCACGGTTAGTC		

Table 3: Temperature and time conditions of the two primers during PCR

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
Pseudomonas 16S rDNA	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
Klebsiella gyrA	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 40 sec.	35	72˚C 10 min.
E. coli phoA	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
qnrS	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
blaTEM	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
blaSHV	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 40 sec.	35	72˚C 10 min.
qepA	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 40 sec.	35	72˚C 10 min.
Proteus atpD	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
Enterobacter gyrB	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.

B. 4. Histopathological examination:

The uterine tissue samples are obtained for histopathological examination after ovariohysterectomy (OHE). The samples are soaked with saline and then rinsed in 10% formalin for 24 hours. After being sectioned, embedded in paraffin, and stained with hematoxylin and eosin, the sample is viewed under a light microscope at varying magnification levels to check for morphological abnormalities brought on by pyometra.

Statistical analysis:
Data was analyzed using a statistical software program (GraphPad Prism for Windows, version 5.01, 2007). Fisher's exact test was used with a $P<0.05$, which is considered statistically significant.

RESULTS

1. Bacterial isolation and identification:
Samples were collected, isolated and the grown colonies were identified after being subjected to biochemical tests, including oxidase, catalase, and urease.
From 17 affected queens, *E. coli* was isolated from 8 of them (47 percent), *Enterobacter* was isolated from 2 of them (11.8 percent), and *Klebsiella* was isolated from only one (5.9 percent). Six of them have shown no growth on the media (35.3 percent) (Tables 3 and 4).
From 8 affected bitches, *E. coli* was isolated from 3 of them (37.5 percent), *Klebsiella* was isolated from one (12.5 percent), and *Pseudomonas* was isolated from one (12.5 percent), while 3 of them showed no growth on the media (37.5 percent) (Tables 4 , 5).

Table 4: Biochemical tests for the identification of bacteria in 17 queens and 8 bitches:

Bacterial isolates	Catalase	Oxidase	Urease
E. coli	Positive (+ve)	Negative (-ve)	Negative (-ve)
Klebsiella	Positive (+ve)	Negative (-ve)	Positive (+ve)
Enterobacter	Positive (+ve)	Negative (-ve)	Negative (-ve)

Table 5: The isolated bacteria from canine and felines affected with pyometra

Bacteria isolated	Number		Occurrence rate (%)	
	Canine	Feline	Canine	Feline
E. coli	3	8	37.5	47
Klebsiella	1	1	12.5	5.9
Enterobacter	0	2	0	11.8
Proteus	1	0	12.5	0
No growth	3	6	37.5	35.3

3. Antimicrobial sensitivity of bacterial isolates:
None of the isolates were sensitive to penicillin antibiotics (including ampicillin (AM) and amoxicillin/clavulanic acid (AMC), cephalosporins (CXM and CFR), or glycopeptides (vancomycin, VA). Only one isolate (7.14%) was sensitive to ciprofloxacin (CIP), while two were intermediate, and 11 isolates were resistant. Of 14 isolates, 12 were resistant to ampicillin/sulbactam (A/S), while the other two isolates were sensitive (14.28%). Three of the 13 isolates (23%) were sensitive to gentamycin (CN), six were intermediate, and the other four were resistant. Five isolates were sensitive (83.3%) to doxycycline (DO), while only one isolate was resistant. Of 13 isolates, eight (61.5%) were sensitive to cotrimoxazole (COT), four were intermediate, and only one isolate was resistant (Figure 1).

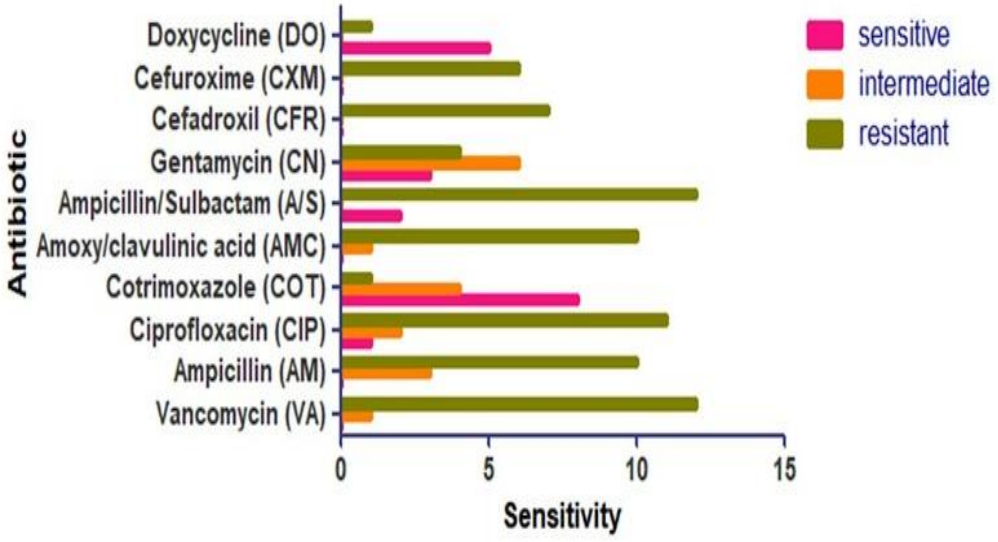

Fig. 1: Antibiotic sensitivity for bacteria isolated from the uterus affected with pyometra.

4. Antibiotic resistance genes among E. coli isolates:

For E. coli isolates isolated from the uterus of queens affected with pyometra, the antibiotic resistance genes detected were as follows: blaTEM and *blaSHV* for the beta-lactamases group and *qnrS* for the quinolones group, while the gene *qepA* was negative (Figure 2-7).

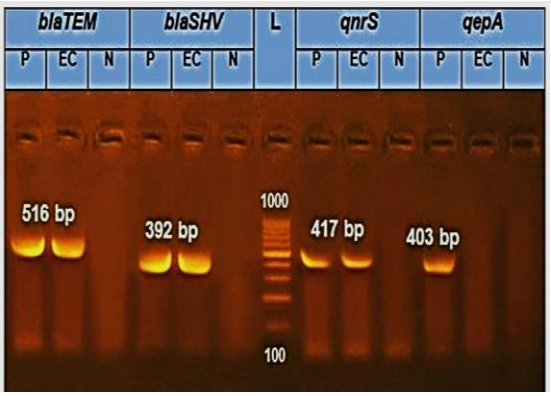

Fig. 2: Agarose gel electrophoresis of *blaTEM*, *blaSHV*, *qnrS*, and *qepA* genes in E. coli isolated from the uterus of bitches and queens affected with pyometra. The band appeared at the expected target size of 516 bp, 392 bp, 417 bp, and 403 bp, respectively. L: ladder, P: positive control, N: negative control.

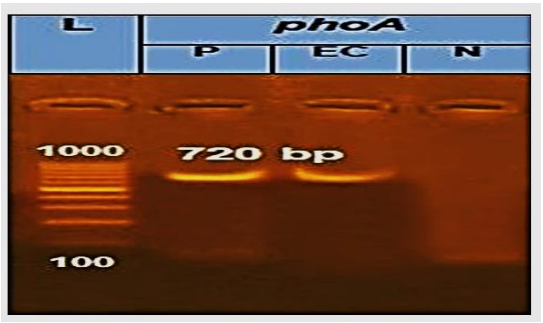

Fig. 3: Agarose gel electrophoresis represents a clear band at 720 bp of E. coli isolated from the uterus of affected bitches and queens with pyometra. L: ladder, P: positive control, N: negative control, EC: positive E. coli.

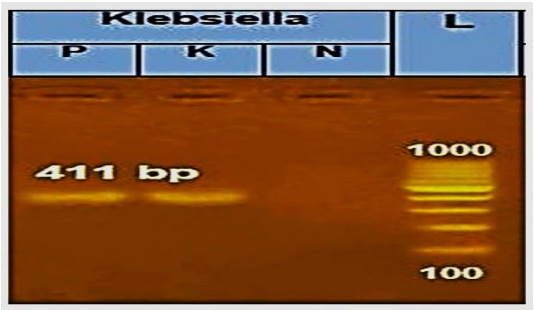

Fig. 4: Agarose gel electrophoresis represents a clear band at 411 bp of Klebsiella isolated from the uterus of affected bitches and queens with pyometra. L: ladder, P: positive control, N: negative control, K: positive Klebsiella

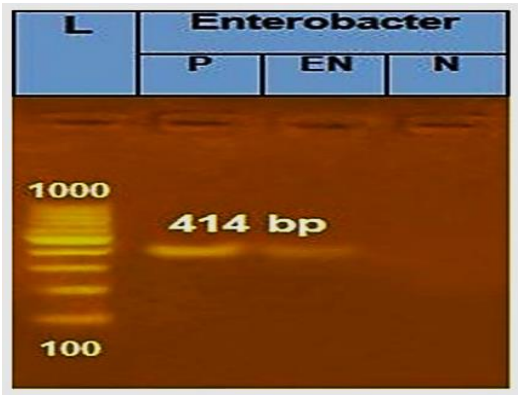

Fig. 5: Agarose gel electrophoresis represents a clear band at 414 bp of Enterobacter isolated from the uterus of affected queens with pyometra. L: ladder, P: positive control, N: negative control, EN: positive Enterobacter

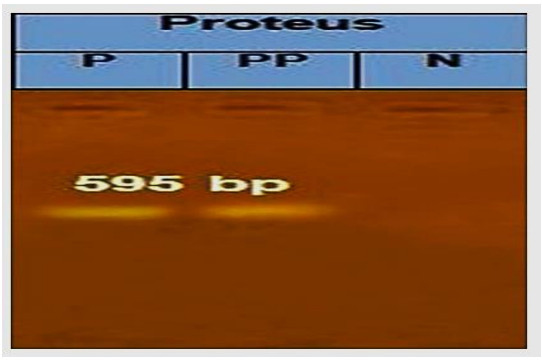

Fig. 6: Agarose gel electrophoresis represents a clear band at 595 bp of Proteus isolated from the uterus of affected bitches with pyometra. P: positive control, N: negative control, PP: positive Proteus

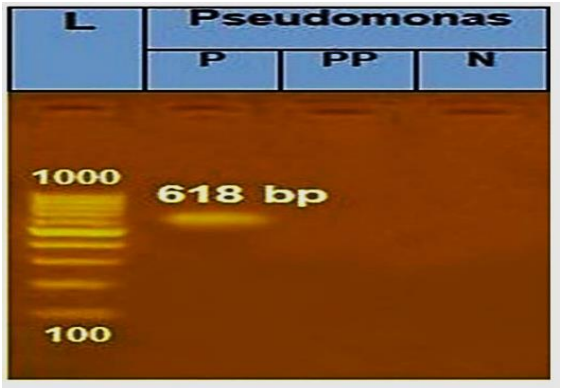

Fig. 7: Agarose gel electrophoresis represents a negative result of pseudomonas where no visible bands or DNA fragments are present. L: ladder, P: positive control, N: negative control, PP: negative pseudomonas.

Histopathological findings:

From 15 samples involved in the study, four infected bitches and seven queens showed the cystic endometrial hyperplasia pyometra complex characterized by the hyperplasia of luminal epithelial cells. Heavy lymphocytic cell infiltration was found in six queens and five bitches. While hemorrhage with the presence of blood in the uterine lumen was present in three queens and one bitch. Also, two queens showed endometrial intraepithelial neoplasia characterized by atypical hyperplasia of irregular cystically dilated glands. Ulceration of the luminal epithelial cells appeared in two queens and three bitches (Fig. 8).

DISCUSSION

In this study, 47% of isolated bacteria from the uterus of affected queens were *E. coli*, while *Enterobacter* represented 11.8% and *Klebsiella* represented 5.9%. In affected bitches, *E. coli* was also the most isolated bacteria, as it represented 37.5%, *Klebsiella* represented 12.5%, and *Proteus* represented 12.5%. These results in bitches were close to previous studies (Sandholm *et al.,* 1975), (Hagman *et al.,* 2009) and (Hagman, 2018)).

The high percentage of *E. coli* may be attributed to the fact that *E. coli* is part of the vaginal microflora of queens and bitches, which easily migrate to the uterus during the diestrus phase ((Tsumagari *et al.,* 2005)). Furthermore, *E. Coli* is a common resident of the gastrointestinal and lower urinary tracts and can enter through the cervix, being contaminated by feces during estrus, followed by bacterial proliferation during the diestrus under the influence of progesterone (Ortega-Pacheco *et al.,* 2012). Also, *E. coli* has a high affinity for progesterone-sensitized endometrium (Fransson *et al.,* 1997). Following that, microbial products, inflammatory mediators, and cytokines induce endometritis (Foster, 2005).

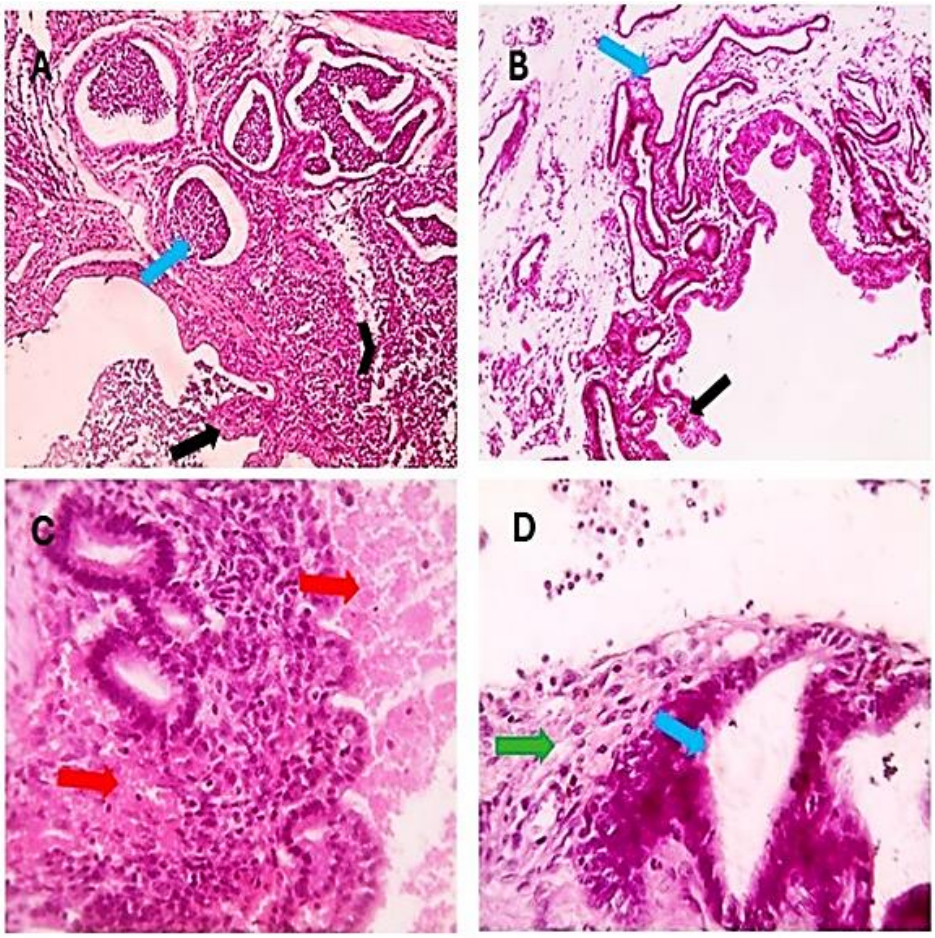

Fig. 8: Microscopic pictures of H&E-stained uterine sections involved in the study showing hyperplasia of luminal epithelial cells (black arrows), irregular cystically dilated glands (blue arrows), and many lymphocytic cell infiltrations in the stroma (arrowheads), endometrial hemorrhage (red arrows), and endometrial fibrosis in four affected queens (green arrows).

In this study, 35.5% of the isolates from queens showed no growth, while in bitches, the percentage was 37.5%. The absence of growth could result from the early administration of antibiotics before surgery or from the misdiagnosis of pyometra with CEH linked to mucometra, hydrometra, and hematometra, which are often sterile (De Bosschere *et al.,* 2001).

Regarding the sensitivity test, the results revealed that Doxycycline (DO) and Cotrimoxazole (COT) were the most effective antibiotics on bacterial isolates, representing 83.3% and 61.5% respectively. These results were in accordance with the results of (Paudel *et al.,* 2023) as they found that both Doxycycline and Cotrimoxazole represented 78% of CLSI-recommended bacterial strains. Ciprofloxacin (CIP), Ampicillin/Sulbactam (A/S), and gentamycin (CN) sensitivity percent were 7.14%, 14.28%, and 23% respectively. Results of the current study revealed that none of the isolates were sensitive to penicillin antibiotics (Ampicillin and Amoxicillin/Clavulanic acid), □ephalon-sporins (Cefuroxime and Cefadroxil), or Vancomycin, and these results are consistent with (Ganiere *et al.,* 2005) and (Ali *et al.,* 2023). Previous studies found that amoxicillin, amoxicillin-clavulanate, and cephalosporins, the most prescribed antibiotics for pyometra cases, showed resistance (Lilenbaum *et al.,* 2000); (Perreten *et al.,* 2010); (Fieni *et al.,* 2014)and (Kim *et al.,* 2018). So, it is not recommended to use these medications as first-line antibiotics for bitches and queens suffering from pyometra. In contrast, Liao

et al, (2020) found that lincomycin was the most resistant antibiotic, representing 96.8%, followed by penicillin (85.5%). Also, Tetracycline has the highest rate of resistance (80%), followed by doxycycline (70%) (Bassessar *et al.,* 2013). These differences may be attributed to the frequency of antimicrobial use and the time of sample collection.

It was found that the resistance genes, blaTEM and blaSHV, made E. coli resistant to the beta-lactam group of antibiotics. Other resistance genes were detected by (Santos, 2006), who found *blaCMY* codes resistance for ampicillin, amoxicillin-clavulanate, cefazolin, ceftriaxone, imipenem, and aztreonam; *blaSIM* for ampicillin, cefepime, imipenem, and aztreonam; and *blaSPM* for ampicillin, cefepime, imipenem, and aztreonam. Antimicrobials like ampicillin, cefepime, or clavulanate induce chromosomes in a variety of microorganisms, primarily in the enterobacteria group, which includes *E. coli*, *Enterobacter spp.*, and *Citrobacter spp*. (Rocha *et al.,* 2021). *qnrS* gene, which can encode resistance to the quinolones, was also detected in the current study.

In terms of histopathology, the inflammatory response was linked to either acute or chronic inflammatory reactions, which were defined by mixed inflammatory cell infiltration of the endometrial stroma. These inflammatory cells comprised neutrophils and lymphocytes. These results are consistent with those of (Gifford *et al.,* 2014) and (Ali *et al.,* 2023). In their studies, they found that many endometritis cases showed a mix of two types of inflammatory cells: neutrophilic polymorphonuclear and mononuclear.

The cystic endometrial hyperplasia-pyometra complex was found in about 27% of the collected samples, characterized by the hyperplasia of luminal epithelial cells. These results agreed with those of (De Bosschere *et al.,* 2001); (Foster, 2005); (Schlafer and Gifford, 2008) and (Verstegen *et al.,* 2008) who discussed how these two lesions (pyometra and cystic endometrial hyperplasia) frequently coexist. Cystic endometrial hyperplasia typically results from inflammatory responses, stromal fibrosis, and endometrial gland deformity (De Bosschere *et al.,* 2001). Some authors revealed that cystic endometrial hyperplasia is the initial stage before pyometra (De Bosschere *et al.,* 2002); (Foster, 2005) and (Pretzer, 2008). However, cystic endometrial hyperplasia has not always developed before pyometra (De Bosschere *et al.,* 2001).

In some cases included in the study, ulceration of the luminal epithelial cells appeared, and this may be due to the microbial agents' persistence, necrotic cells, and neutrophil infiltration (Kumar *et al.,* 2015). Endometrial atrophy may be related to age, as the level of follicle-stimulating hormone (FSH) is high. FSH may cause uterus atrophy by inhibiting proliferation and promoting apoptosis of endometrial glandular cells (Zhang *et al.,* 2015). The prolonged use of estrus suppression medicines can be the cause (England and Heimendahl, 2010).

CONCLUSION

Pyometra is considered a life-threatening disease that must be early diagnosed, using diagnostic tools for early assessment of the case to determine the best treatment options. *E. coli* was found to be the most isolated bacteria in both bitches and queens. It was found that the most effective antimicrobials to be the first line of choice for combating the infection associated with pyometra are Doxycycline and Cotrimoxazole. These results may help choosing the appropriate antimicrobial drugs before surgical intervention or in medical treatment. Early detection and careful monitoring of antimicrobial susceptibility are crucial before therapeutic intervention to decrease the chance of antimicrobial resistance.

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التقييم الميكروبيولوجي والنسيجي لمشاكل تقيح الرحم في الكلاب والقطط

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يعد التقيح الرحمي من المشاكل التي تهدد حياة القطط والكلاب والتي يجب ان تكتشف وتعالج مبكراً حفاظاً على حياتهم. في هذه الدراسة نستعرض أكثر أنواع البكتريا التي تم عزلها من الرحم المصاب واهم المضادات الحيوية التي يجب ان تستخدم للعلاج بناءاً على العزل البكتيري واختبار الحساسية. في هذه الدراسة وجدت بعض الجينات المقاومة لبعض أنواع المضادات الحيوية والتي بدورها تجعل الاستجابة لهذه الأنواع ضعيفة اثناء العلاج لذلك يفضل تجنب استخدام هذه المضادات الحيوية اثناء العلاج. يوجد العديد من التغيرات النسيجية في جدار الرحم المتقيح وتختلف درجات التغيرات من حالة لأخرى. تم تجميع العينات من الحيوانات المصابة بعد إزالة الرحم جراحياً من اجل الفحص النسيجي. اي كولاي هي أكثر أنواع البكتيريا عزلاً في القطط والكلاب. أكثر المضادات الحيوية حساسية هو دوكسيسايكيلين حيث أنه وجد ٨٣,٣٪ من البكتيريا حساسة لهذا المضاد الحيوي. التشخيص المبكر لمشاكل تقيح الرحم والعزل البكتيري المبكر واختيار أفضل مضاد حيوي بناءاً على اختبار الحساسية ضروري من اجل تقليل مقاومة البكتيريا للمضادات الحيوية.