



RESEARCH ARTICLE

Pyometra in Dogs, Clinical, Bacteriological and Histopathological Observations

Othman J Ali^{*1,2}, Ali H Hassan³, Nahla M Saeed⁴ and Talib GM Ali¹

¹Department of Surgery and Theriogenology, College of Veterinary Medicine, University of Sulaimani, Sulaymaniyah, Kurdistan Region, Iraq; ²Department of anesthesia, college of health sciences, Cihan University Sulaimaniya, Sulaimaniya, Kurdistan Region, Iraq; ³Department of Basic Science, College of Dentistry, University of Sulaimani, Sulaymaniyah, Kurdistan Region, Iraq; ⁴Department of Microbiology, College of College of Veterinary Medicine, University of Sulaimani, Sulaymaniyah, Kurdistan Region, Iraq.

*Corresponding author: othman.ali@univsul.edu.iq

ARTICLE HISTORY (23-234)

Received: June 8, 2023
Revised: September 11, 2023
Accepted: September 15, 2023
Published online: October 17, 2023

Key words:

Pyometra
Bitches
Suppurative endometritis
Ovariohysterectomy
OHE

ABSTRACT

Pyometra is a life-threatening uterine infection, accompanied by accumulation of intrauterine purulent exudate. The objective of this study was to show the recent laboratory and histopathological finding of pyometra in dogs with their correlation to their ages, and postoperative outcome. In this study, eleven cases of pyometra out of 115 female dogs of different ages and breeds were admitted to the Veterinary Teaching Hospital/College of Veterinary Medicine/University of Sulaimani from 2017 to 2021 for the purpose of ovariohysterectomy (OHE). They were diagnosed according to history, clinical signs, physical examination and the trans-abdominal ultrasound inspections. The uterine content and biopsy were collected during OHE for bacteriological and histopathological study respectively. The pyometra-uteri were plated on blood agars for 24 hours. The growing bacterial colonies were inoculated on Mannitol Salt, MacConkey and Eosin Methylene Blue (EMB) agar, and their strains were determined using the VITEK 2 system. In the results, a significant difference was found between the cases of pyometra and the normal bitches, the average age of the pyometra-dogs was 265 months old (± 176.56 STD), while the average age of non-pyometra bitches was 23 months old (± 15.64 STD). *Escherichia coli* and *Staphylococcus cohnii* were recovered from the infected uterus. Chronic suppurative endometritis or metritis were identified, which associated with purulent and mucopurulent inflammatory exudates in the uterine lumen, focal epithelial sloughing, endometrial hyperplasia and fibrosis, cystic dilatation of the endometrial gland and infiltration of the endometrial stroma by mixed inflammatory reaction consisting of mononuclear and neutrophilic polymorphonuclear inflammatory cells. It was concluded that pyometra was an age-related infection and the periodic reproductive examination should be carried out for prompt surgical treatment.

To Cite This Article: Ali OJ, Hassan AH, Saeed NM and Ali TGM, 2023. Pyometra in dogs, clinical, bacteriological and histopathological observations. Pak Vet J, 43(4): 643-650. <http://dx.doi.org/10.29261/pakvetj/2023.096>

INTRODUCTION

Pyometra is a serious female reproductive disease that is characterized by intrauterine accumulation of purulent exudate and persistent corpus luteum. It usually affects middle and old-aged female dogs, particularly in the diestrus phase of their estrous cycle; however, it has also been observed in young bitches of few months old after their first heat cycle (Kempisty *et al.*, 2013; Porowska *et al.*, 2018; Hagman, 2018). As well as, it has been reported that female dogs that have never given birth are particularly susceptible to pyometra (Kim and Kim, 2005; Smith,

2006). In addition, certain breeds possibly increase the likelihood of developing pyometra such as the Rough Collie, Rottweiler, Cavalier King Charles Spaniel, and Golden Retriever (Egenvall *et al.*, 2001).

The pathogenesis of pyometra is not completely understood, however, it is deemed to be initiated by hormonal disturbances, particularly progesterone hormone, which then complicated by secondary bacterial infection (Porowska *et al.*, 2018). Progesterone hormone causes a significant decrease of the local uterine defense mechanism and giving an opportunity for bacterial infection that stimulates uterine glands secretions. Uterine secretions

could be trapped within the uterine lumen particularly when the diestrus phase is preceded by an elongated heat cycle in association with estrogen-producing ovarian cyst that induce endometrial proliferation (Kida *et al.*, 2006; Kempisty *et al.*, 2013). *Escherichia coli* is commonly isolated from canine pyometra (Hagman, 2012; Mateus *et al.*, 2013), as well as, *Enterobacter cloacae*, *Streptococcus canis*, *Klebsiella spp.*, *Proteus spp.*, and *Pseudomonas spp.* had also been isolated in some cases, however, in few studies bacterial infections were not identified (Porowska *et al.*, 2018). The presence of these bacterial infection leads to endotoxic shock and removing the infection uteri is considered the key point to prevent the animals from endotoxic shock. Therefore, ovariohysterectomy (OHE) should not be postponed in order to cease the risk of sepsis and endotoxemia. Up to date, surgical OHE is still considered the most effective treatment than the moderate medication (Hagman, 2022). This study aimed to show the current laboratory and histopathological finding of pyometra in dogs with their correlation to their breeds, ages, as well as the goal of surgical success and postoperative outcome.

MATERIALS AND METHODS

Animals: This study was conducted from 2017 to 2021, on the 115 bitches were admitted into the Veterinary Teaching Hospital of the College of Veterinary Medicine, University of Sulaimani, for the purpose of OHE. During the study period, eleven cases of pyometra were diagnosed according to their case history, clinical signs, physical examination, and trans-abdominal ultrasound (SIUI 5500, China). The remaining 104 bitches were undergoing routine OHE without any abnormal clinical conditions (Table 1).

Ethical approval: All the procedures and approaches of this study were conducted and approved according to the principles of the ethics by the college of the veterinary medicine research committee, University of Sulaimani, Kurdistan Regional Government, Kurdistan/ Iraq.

The data were collected and studied from the Department of Surgery and Theriogenology at the veterinary teaching hospital and analysed in accordance with the other recorded retrospective clinical studies in agreement with an authorized institution.

Anesthetic technique: Anesthesia was made by subcutaneous injection of Atropine Sulphate, a premedicated drug, at a dose of 0.04 mg/kg. Twenty to thirty minutes later, followed by intramuscular injection of Xylazine 2% (Interchemie werken) and Ketamine 10% (Alfasan), at a dose of 1 mg/kg and 15 mg/kg, respectively.

Surgical procedure: The ventral abdominal wall was prepared for an aseptic operation from the xiphoid to the pubis and bilaterally as far as the flanks. After placing the animal on the dorsal recumbency, the hair was clipped, shaved, and the skin was scrubbed with a 2.5% tincture of iodine and draped with surgical drapes.

An incision through the ventral abdominal wall was done over the middle third of the distance between

umbilicus and pubis. The spay hook was utilized to locate and exteriorize the uterine horns. A clamp was placed on the proper ligament and the ovary was gently retracted out of the abdomen by rupturing the suspensory ligaments by the aid of the index finger. Two mosquito hemostatic forceps were placed across the ovarian pedicle below the ovary. The pedicle was ligated with one or two absorbable suture ligatures 2-0 or 3-0, and then the pedicle transected between the clamps, and the similar procedure was repeated for the adjacent ovary. Then the uterine horns were localized by tearing of the broad ligaments by the aids of gauze or haemostatic forceps. The uterine body was ligated with one or two transfixing ligatures of 0 or 1 absorbable ligature. The abdominal wall musculature closed with 2-0 or 3-0 absorbable monofilament suture in a simple continuous pattern. The sutures were placed through a full thickness of the rectus abdominal muscles. Finally, the skin apposed with an intradermal pattern, using non absorbable sutures.

Postoperative care: Antibiotic and analgesic medicines were injected in order to prevent postoperative pain, infection, and complications. Animal owners were advised to use head collar in order to protect the abdominal wound from licking and postoperative wound dehiscence. Skin sutures were removed after 12 days of the operation.

Bacteriological isolation and identification: The samples were collected aseptically from the uterine content during surgical operation. Pyometra samples were plated on the blood agar at 37°C for 24 hours. After complete growth, the selected colonies were identified and differentiated by inoculating on the Mannitol Salt Agar, MacConkey agar and Eosin Methylene Blue (EMB) agar in order to identify both the gram-positive and gram-negative bacteria (Winn Washington and Elmer Koneman, 2006). Then after confirmation of the presence of these characteristics feature of the colonies, the isolated bacteria were identified (Cheesbrough, 1981; Quinn *et al.*, 2002); and finally their strains were determined using the VITEK 2 system according to the manufacturer's instructions (Bannerman *et al.*, 1993). Further, antimicrobial susceptibility to antimicrobial agents were performed by the agar disc diffusion method on Mueller Hinton Agar (Himedia, Mumbai, India) incubated at 37°C for 24 hrs (Bauer *et al.*, 1966).

Histopathological preparation and examination: The uterine tissue samples were prepared for histopathological examination according to (Suvarna *et al.*, 2019). Briefly; The obtained tissue samples were cleaned with normal saline, fixed in 10% neutral buffer formalin for 24 hours, washed properly with tap water, dehydrated in ascending concentrations of ethanol alcohol, cleared by two successive immersions in xylene, and impregnated and embedded in paraffin. Following that, 5 µm-thick tissue sections were obtained using a rotary microtome, stained with hematoxylin and eosin, then examined using different magnifying power of light microscopy to assess the morphological characteristics of the infected uterine tissue.

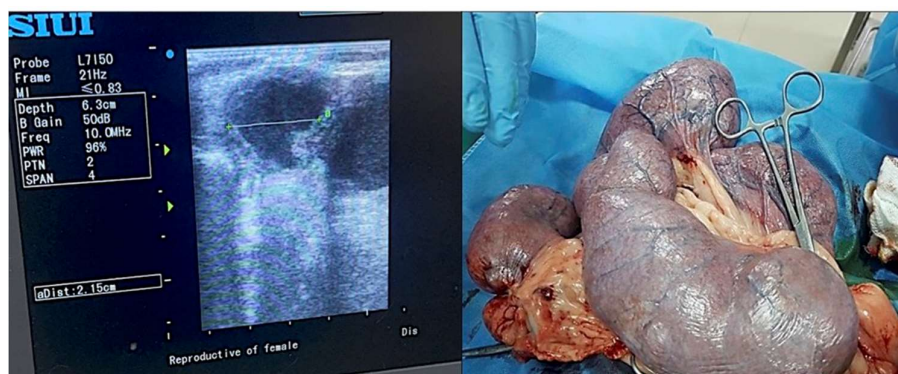


Fig. 1: Showing ultrasonographic image of the uterine horns with low echogenic materials (Fluids) before the operation, and sever distended uterine horns in a very advanced case of closed pyometra during the operation.

Table 1: Shows the the affected breeds, ages, and their prognosis of dogs suffered from pyometra after treatment with OHE.

No.	Affected Breeds	Ages/ Years	Treatment	Prognosis
1	German Shepherd	12	OHE	Survived
2	German Shepherd	8	OHE	Survived
3	Chow Chow	7	OHE	Not-survived/ Passed after 24 hours from endotoxic shock
4	Maltese	2.5	OHE	Survived
5	Maltese	10.5	OHE	Survived
6	Maltese	11	OHE	Survived
7	Maltese	12	OHE	Survived
8	Maltese	12	OHE	Survived
9	Maltese	14	OHE	Survived
10	Shih Tzu	12	OHE	Survived
11	Anatolian Shepperd	9	OHE	Survived

Statistical analysis: The results were presented as mean \pm standard deviation (SD) using GraphPad Prism software (version 9) for statistical analysis, un-paired T test was used with a ($P < 0.05$) was considered statistically significant.

RESULTS

Clinical findings: Generally, the observed clinical signs were fever, loss of appetite, depression, cessation of the estrous cycle, and distended abdomen. In open pyometra there were intermittent mucopurulent to a hemorrhagic vaginal discharge. Nevertheless, the clinical signs were disappeared for a few days after each treatment regimens with antibiotics and anti-inflammatory agents, which then followed by recurrent episodes of pyometra after stopping the treatments.

Following the surgical operation, the engorged uterine horns were exteriorized on the surgical site. The walls of the uterine horns were distended, congested, turgid and filled with fluids that were varied in color ranged from yellow to red. The sizes or diameter of the distended uterine horns were also varied in different clinical cases. In the advanced closed pyometra the diameter of the infected uterine horns were approximately reached up to 9 cm (Fig. 1). In few cases, the uterine exudate was ascended through the fallopian tubes and produced abscesses within the ovarian parenchyma.

Statistically, it was found that the age was the main contributing factor of pyometra. The average ages at which the dogs were suffering from pyometra was 265 months old (± 176.56 STD), with a minimum and maximum age of 30 and 168 months respectively. However, in the control cases from normal dogs (non-pyometra) who underwent surgery, the recorded average age was 23 months old (± 15.64 STD) with the minimum and maximum ages of 7 months and 108 months, respectively (Fig. 2).

The overall proportion of pyometra disease that developed in different breeds of bitches was 9.5%, however, there were no breed predisposition. Ten cases out of the total eleven pyometra cases were recovered after the surgical treatment, and the last case was died from endotoxemic shock 24 hours after the operation. Therefore, the rate of success in using surgical OHE for the treatment of pyometra in this study was 90.9%.

Bacteriological isolation and identification: Samples of all cases of pyometra were analyzed for the isolation and identification of bacteria. Based on the microbiological and biochemical tests, *Escherichia coli* was isolated, which was a gram-negative coccus that produced pink colony on MacConkey agar and metallic sheen colony on EMB agar. As well as, *Staphylococcus coagulase-negative* was also isolated, which was a gram-positive coccus, that had small pink or red colonies with no colour change on the Mannitol salt agar. Further, the strain was identified as *Staphylococcus cohnii* sub spp. urealyticus using the VITEK 2 system. The results of sensitivity test showed that *E. coli* and *Staphylococcus cohnii* sub-spp. urealyticus were resistance to methicillin, amoxycillin, penicillin G, ampicillin, cefuroxime, and vancomycin.

Histopathological findings: Chronic suppurative endometritis was observed in ten out of the eleven total pyometra-uteri that involved in this study. Purulent and mucopurulent inflammatory exudates were seen in the uterine lumen and in some of the endometrial glands associated with focal epithelial sloughing and infiltration of the endometrial stroma by a mixed inflammatory reaction consisting of mononuclear and neutrophilic polymorphonuclear inflammatory cells (acute on chronic inflammation). Endometrial fibrosis and hyperplasia were

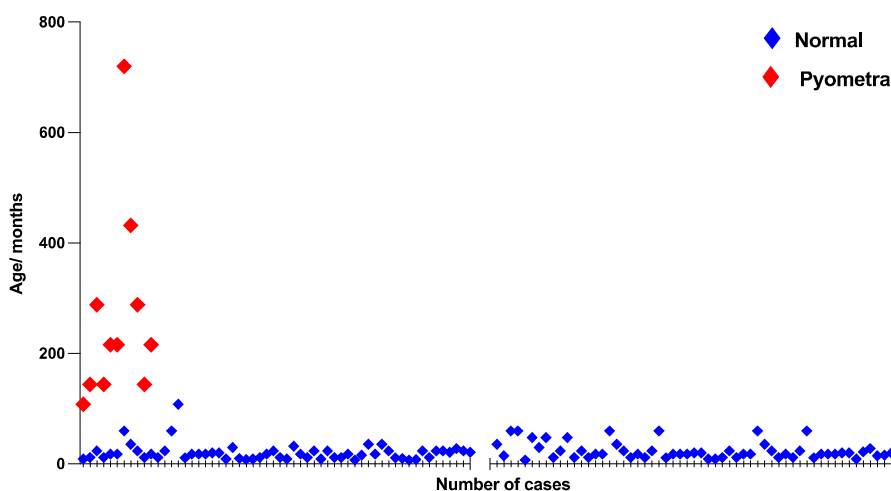


Fig. 2: Shows distribution of normal (non-pyometra) and cases of pyometra in relation to their ages that reflect a significant contributing factor of pyometra, using GraphPad prism version 9.3.1.

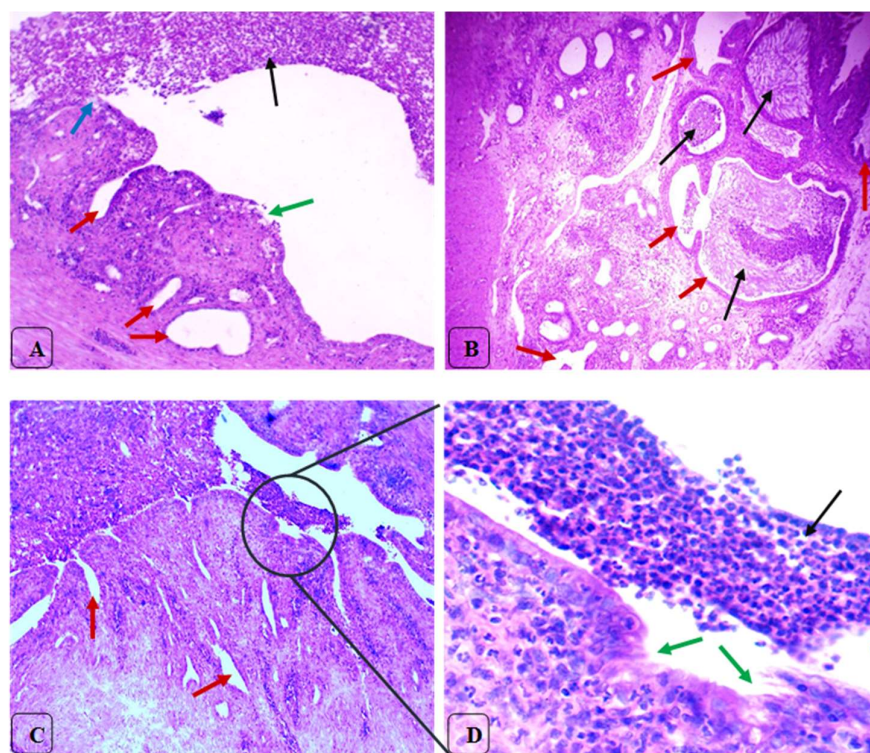


Fig. 3: Light microscopy of pyometra-uteri involved by this study showing chronic suppurative endometritis with an intense purulent or mucopurulent exudate within the uterine lumen and within the endometrial glands (black arrows), focal epithelial sloughing (green arrows), cystic hyperplasia of the endometrial glands (red arrow) which appear convoluted rather than straight and infiltration of the endometrial stroma by mixed inflammatory reaction consisting of mononuclear and neutrophilic polymorphonuclear inflammatory cells. H and E, A, B and C X100, D X400.

also evident associated with cystic dilatation of the endometrial gland which appears convoluted rather than straight with formation of variably sized cross sections (Fig. 3 and 4).

In the last case of pyometra-uterus there was an acute on chronic metritis, that was associated with purulent exudate in the uterine lumen, endometrial fibrosis, and infiltration of the endometrium, myometrium and perimetrium by a mixed inflammatory reaction of mononuclear and neutrophilic polymorphonuclear inflammatory cells (Fig. 5). Furthermore, there was a marked reduction in thickness of the endometrium, a

decrease in number of endometrial glands and infiltration of the endometrial stroma by mononuclear inflammatory cells (Fig. 6).

DISCUSSION

Pyometra is a life-threatening disease of the female genitalia in dogs with a subsequent reported complications including septic shock, peritonitis, and disseminated microbial multi-organ dysfunction (Kempisty *et al.*, 2013; Jitpean *et al.*, 2014). In a retrospective study on 200,000 dogs, it was reported that 24% they were affected with

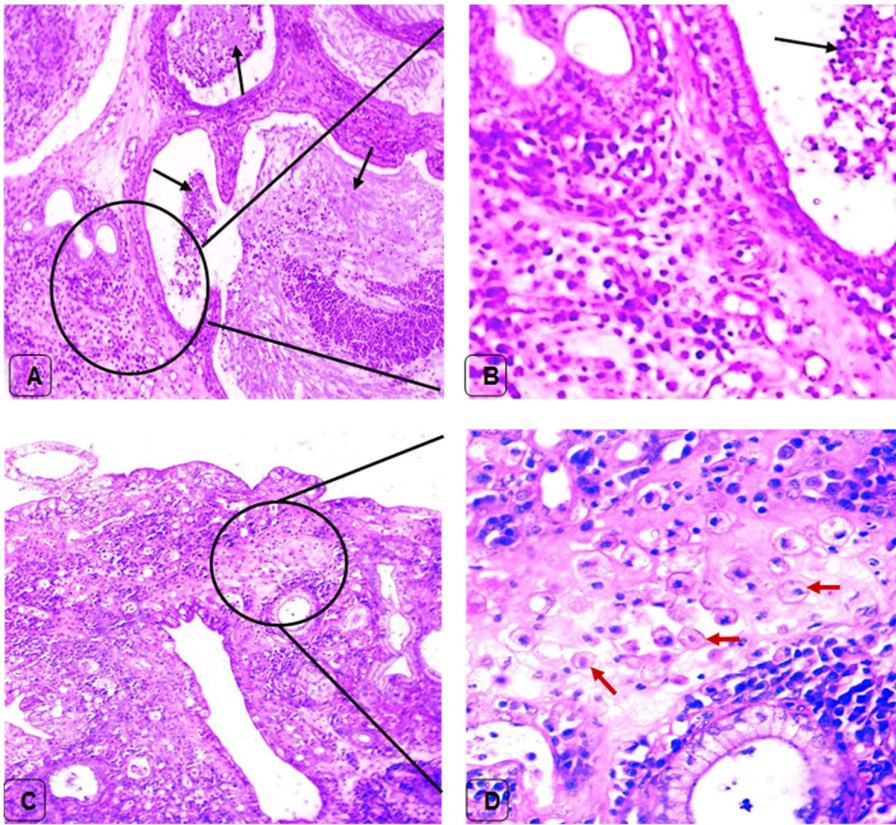


Fig. 4: Light microscopy of pyometra-uteri involved by this study. A and B: Marked cystic endometrial hyperplasia associated with the presence of mucopurulent exudate within the endometrial glands (black arrows) and infiltration of the endometrial stroma by mononuclear inflammatory cells, mainly lymphocytes and plasma cells. C and D: Cystic endometrial hyperplasia associated with infiltration of the endometrial stroma by mononuclear inflammatory cells mainly lymphocytes, plasma cells, and foamy macrophages (red arrows). H and E, A and C X200, B and D X400.

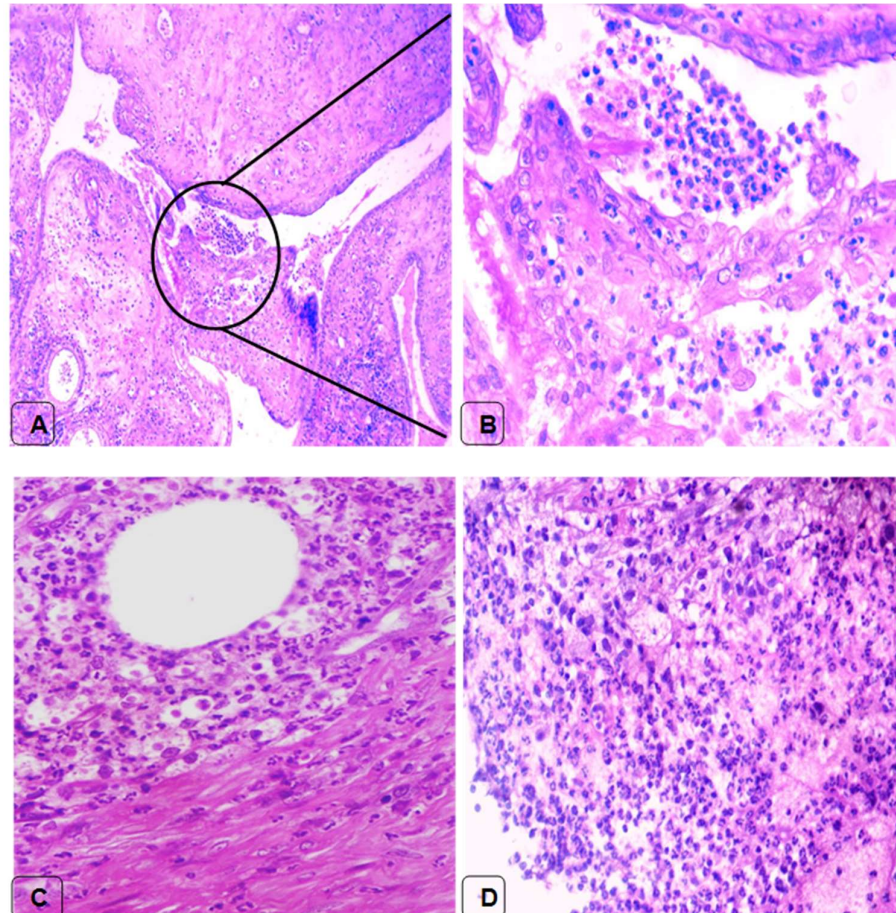


Fig. 5: Light microscopy of a pyometra-uterus showing chronic suppurative metritis. A and B: Purulent exudate consisting of pyknotic neutrophils & necrotic debris in the uterine lumen associated with endometrial hyperplasia and infiltration of the endometrial stroma by mixed inflammatory reaction consisting of mononuclear and neutrophilic polymorphonuclear inflammatory cells. C: Cystic dilatation of an endometrial gland associated with infiltration of the deep endometrial connective tissue stroma and myometrium by mononuclear and neutrophilic polymorphonuclear inflammatory cells. D: An intense infiltration of the perimetrial connective tissue stroma by mononuclear and neutrophilic polymorphonuclear inflammatory cells. H and E, A X100, B, C and D X400.

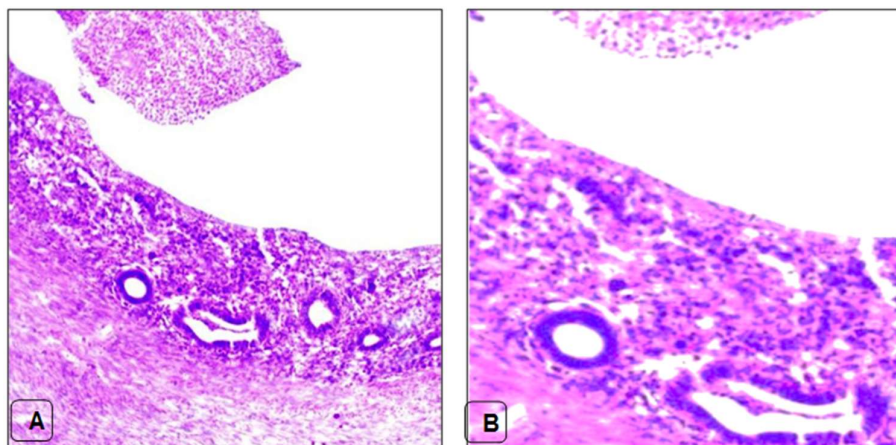


Fig. 6: Light microscopy of a uterus with pyometra showing chronic suppurative endometritis and endometrial atrophy. A mucopurulent exudate is evident in the uterine lumen and one of the endometrial glands associated with endometrial fibrosis, marked reduction in the endometrial thickness, a decrease in number of the endometrial glands, and infiltration of the endometrial stroma by mononuclear inflammatory cells. H and E, A, X200, B, X400.

pyometra before they turned on 10 years old (Egenvall *et al.*, 2001). Although, in this study, it was found that the average ages of the affected bitches with pyometra was 10 years, were 36% under 10 years and 64% beyond 10 years old. When compared to the normal cases of OHE, this finding indicates that age was considered the main predisposing factor for bitches to become more susceptible for pyometra, were the average ages of normal bitches was 1.9 years old. However, the proportion pyometra could be varied and unstable according to their different predisposing factors, and it was recorded in different studies that regular pregnancy had a protective effect against pyometra (Hagman *et al.*, 2011; Jitpean *et al.*, 2014). Therefore, regular inspection and controlling of the predisposing factors could aid the dogs to be prevented from contracting pyometra.

According to this study, it was shown that *E. coli* and *Staphylococcus cohnii* sub-ssp. *urealyticus* constitute a significant component of the normal microflora that implicated in the development of endometritis, metritis and finally formation of the inflammatory exudate. These bacteria are opportunistic pathogens, and their pathogenesis in the formation of canine pyometra is still unclear and needs further investigation. Although, the animals involved in this study were not hospitalized, because long-term hospitalization is considered to be one of the predisposing factor for the opportunistic microorganisms to boost pyometra (Lilenbaum *et al.*, 1998). Isolation and identification of these bacteria depend on using different media such as MacConky and EMB agar for *E. coli* and Mannitol salt agar for *Staphylococcus* species, which they showed specific colony characteristics according to the literatures that agreed with (Soldera *et al.*, 2013). For antimicrobial susceptibility, these bacteria had strong resistance to methicillin, Amoxicillin, penicillin G, ampicillin, cefuroxime, and vancomycin. Resistance to B-lactam antibiotic it has been similarly demonstrated recently that 62% of the isolates of *Staphylococcus* strains of canine pyoderma origin were b-lactamase producers (Ganiere *et al.*, 2005). The occurrence of b-lactamase producers in this genus has substantially grown compared to a previous study of a similar canine population, although, it was sensitive to enrofloxacin, cefuroxime, gentamycin, erythromycin, ciprofloxacin, doxycycline, trimethoprim/sulphamethoxazol, chloramphenicol, and neomycin (Lilenbaum *et al.*, 2000; Perreten *et al.*, 2010). These

antimicrobial susceptibilities allow us to understand their different degrees of resistance to those antibiotics commonly used in veterinary medicine and they were useless for the treatment of pyometra.

Chronic suppurative endometritis or metritis was evident in ten out of the eleven pyometra-uteri involved in this study, whereas the last pyometra-uterus was suffering from chronic endometritis and endometrial atrophy. In all these eleven cases, the inflammatory response was associated with acute on chronic inflammatory reactions that were characterized by infiltration of the endometrial stroma by mixed inflammatory cells consisting of mononuclear and neutrophilic polymorphonuclear inflammatory cells. Acute on chronic inflammation is a chronic inflammatory lesions that continue to show infiltration of neutrophils due to persistence of the causative microbial agents and/or the necrotic cells or due to elaboration of neutrophils-attracting inflammatory mediators by macrophages (Kumar *et al.*, 2013). This finding is in agreement with that of Gifford *et al.* (2014) who stated that many of the endometritis cases involved in their study showed a mixed inflammatory reaction of mononuclear and neutrophilic polymorphonuclear inflammatory cells. The focal epithelial sloughing that were seen in association with these pyometra-uteri is possibly attributed to persistence of the causative microbial agents, necrotic cells, and neutrophil infiltration (Kumar *et al.*, 2013).

Presence of the purulent and mucopurulent exudate in the uterine lumen and in some of the endometrial glands was associated with cystic endometrial hyperplasia. This result agrees with the findings of many other authors (De Bosschere *et al.*, 2001; Foster, 2005; Schlafer and Gifford, 2008; Verstegen and Verstegen, 2008) who refer to the frequent combination of these two lesions (pyometra and cystic endometrial hyperplasia). Cystic endometrial hyperplasia-pyometra complex is taking place as a result of bacterial and hormonal interaction. Ascending bacterial infection usually occurs in the proestrus or oestrus phases, followed by bacterial proliferation during the dioestrus under the influence of progesterone. Subsequently, microbial products and/or inflammatory mediators and cytokines induce endometrial inflammation (Foster, 2005). Cystic endometrial hyperplasia usually develop because of cystic distortion of endometrial glands, stromal fibrosis and inflammatory reaction (De Bosschere *et al.*, 2001) and it

has been considered by some authors to be the preliminary stage in the occurrence of pyometra (De Bosschere *et al.*, 2002; Foster, 2005; Pretzer, 2008). However, pyometra has not always been preceded by cystic endometrial hyperplasia (Nomura and Funahashi, 1999; De Bosschere *et al.*, 2001). Endometrial atrophy and chronic endometritis were seen in ten pyometra-uterus involved in this study. This finding, can be ascribed to the severe and progressive tissue damage and fibrosis that often accompanied by chronic inflammatory lesions (Kumar *et al.*, 2013), is in agreement with that of (De Bosschere *et al.*, 2001) who observed, in their study of 42 bitches with a clinical suspicion of pyometra, hyperplasia or atrophy of the endometrium, associated with cystic changes of the endometrial glands and it also in agreement with that of (Zanghi *et al.*, 2007) who noted a diffuse chronic endometritis and marked atrophy of the endometrium in a pyometra-bitch with endometrial polypoid adenomyomatosis and ovarian granulosa cell tumour. Endometrial atrophy could be an age-related event due to the high level of follicle stimulating hormone (FSH), which can induce atrophy of the uterus by inhibiting proliferation and promoting apoptosis of the endometrial glandular cells (Zhang *et al.*, 2015) or it can attributed to the pronged use of oestrus suppression drugs (England and Angelika 2010). However, in many instances, the causes of endometrial atrophy are still unknown (Bakour *et al.*, 2000; Senturk and Erel, 2008).

Conclusions: Periodic reproductive examination and detection of pyometra should be carried out, using useful diagnostic tools for determining the predicted outcome and the early surgical treatment, because pyometra is considered a life-threatening disease that associated with subsequent complications such as endotoxic shock, and disseminated microbial multi-organ dysfunction such as isolated *E. coli* and *Staphylococcus cohnil* subspp. urealyticus strains were resistant to various antibiotics.

Acknowledgments: We would like to thank the Veterinary Teaching Hospital belonging to the College of Veterinary Medicine, University of Sulaimani, Sulaymaniyah, as well as the private VetGreen hospital for their support in performing all the surgical operations and processing the samples for bacteriological and histopathological examination.

The project is funded by the University of Sulaimani, Ministry of Higher education and scientific research, Kurdistan Regional Government, Kurdistan, Iraq.

Conflict of interest: All authors declare that they have no conflict of interest.

Statement of human and animal rights: All the surgical operations of this study were conducted in conceit with the owners according to the approved principles of the ethics by the college of the veterinary medicine research committee, University of Sulaimani, Kurdistan Regional Government, Kurdistan/ Iraq.

Data availability: The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors contribution: O.J.A involved in the conception of the study, the design of the study, and the execution of the surgical operation in this study. As a co-author of the paper, T.G.M.A contributed to the writing of the introduction, the methods, the discussion, and formatting. A. H.H contributed to the preparation and reading of the histopathological section and finally N.M.S. was contributed in microbiological section of this research, including culturing, sensitivity and VITEK test.

REFERENCES

- Bakour SH, Khan KS and Gupta JK, 2000. Controlled analysis of factors associated with insufficient sample on outpatient endometrial biopsy. *Bjog: An Int J Obst Gynecol* 107:1312-4.
- Bannerman TL, Kleeman KT and Kloos VWE, 1993. Evaluation of the Vitek Systems Gram-Positive Identification card for species identification of coagulase-negative staphylococci. *J Clin Microbiol* 31:1322-5.
- Bauer AW, Kirby WM, Sherris JC, *et al.*, 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 45:493-6.
- Cheesbrough M, 1981. Medical laboratory manual for tropical countries. Vol. 1. M. Cheesbrough, 14 Bevills Close, Doddington, Cambridgeshire, PE15 OTT.
- De Bosschere H, Ducatelle R, Vermeirsch H, *et al.*, 2002. Estrogen-alpha and progesterone receptor expression in cystic endometrial hyperplasia and pyometra in the bitch. *Anim Reprod Sci* 70:251-9.
- De Bosschere H, Ducatelle R, Vermeirsch H, *et al.*, 2001. Cystic endometrial hyperplasia-pyometra complex in the bitch: should the two entities be disconnected? *Theriogenology* 55:1509-19.
- Egenvall A, Hagman R, Bonnett BN, *et al.*, 2001. Breed risk of pyometra in insured dogs in Sweden. *J Vet Intern Med* 15:530-8.
- England GCW and Angelika VH, 2010. Association British Small Animal Veterinary. 2010. BSAVA manual of canine and feline reproduction and neonatology (British Small Animal Veterinary Association: Quedgeley, Gloucester [England]).
- Foster R, 2005. Surgical Pathology of the canine female reproductive tract. URL: http://www.uoguelph.ca/~rfoster/repropath/surgicalpath/female/dog/female_dog.
- Ganiere JP, Medaille C and Mangion C, 2005. Antimicrobial Drug Susceptibility of *Staphylococcus intermedius* Clinical Isolates from Canine Pyoderma. *J Vet Med Series B* 52:25-31.
- Gifford AT, Scarlett JM and Schlafer DH, 2014. Histopathologic findings in uterine biopsy samples from subfertile bitches: 399 cases (1990-2005). *J Am Vet Med Assoc* 244:180-6.
- Hagman R, 2012. Clinical and molecular characteristics of pyometra in female dogs. *Reprod Domest Anim* 47 Suppl 6:323-5.
- Hagman R, 2018. Pyometra in Small Animals. *Veterinary Clinics of North America: Small Animal Practice* 48:639-61.
- Hagman R, 2022. Pyometra in Small Animals 2.0. *Veterinary Clinics: Small Animal Practice* 52:631-57.
- Hagman R, Anne-Sofie L, Åke H, *et al.*, 2011. A breed-matched case-control study of potential risk-factors for canine pyometra. *Theriogenology* 75:1251-57.
- Jitpean S, Pettersson A, Höglund O V, *et al.*, 2014. Increased concentrations of Serum amyloid A in dogs with sepsis caused by pyometra. *BMC Vet Res* 10:273.
- Kempisty B, Dorota B, Magdalena W, *et al.*, 2013. Endometritis and pyometra in bitches: A review. *Veterinari Medicina* 58:289-97.
- Kida K, Baba E, Torii R, *et al.*, 2006. Lactoferrin expression in the canine uterus during the estrous cycle and with pyometra. *Theriogenology* 66:1325-33.
- Kim KS and Kim O, 2005. Cystic endometrial hyperplasia and endometritis in a dog following prolonged treatment of medroxyprogesterone acetate. *J Vet Sci* 6:81-2.
- Kumar V, Abul K, Abbas JC, *et al.*, 2013. Robbins basic pathology (Elsevier/Saunders: Philadelphia, PA).
- Lilenbaum W, Nunes ELC and Azeredo MAI, 1998. Prevalence and antimicrobial susceptibility of staphylococci isolated from the skin surface of clinically normal cats. *Lett Appl Microbiol* 27:224-28.
- Lilenbaum W, Veras M, Blum E, *et al.*, 2000. Antimicrobial susceptibility of staphylococci isolated from otitis externa in dogs. *Lett Appl Microbiol* 31:42-45.

- Mateus L, Henriques S, Merino C, et al., 2013. Virulence genotypes of *Escherichia coli* canine isolates from pyometra, cystitis and fecal origin. *Vet Microbiol* 166:590-4.
- Nomura K and Funahashi H, 1999. Histological characteristics of canine deciduoma induced by intrauterine inoculation of *E. coli* suspension. *J Vet Med Sci* 61:433-8.
- Perreten V, Kadlec K, Schwarz S, et al., 2010. Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. *J Antimicrob Chemother* 65:1145-54.
- Porowska E, Magdalena K, Maurycy J, et al., 2018. Selected aspects of endometritis – pyometra complex in dogs – current troubles and treatment perspectives. *Med J Cell Biol* 6:108-13.
- Pretzer SD, 2008. Clinical presentation of canine pyometra and mucometra: a review. *Theriogenology* 70:359-63.
- Quinn PJ, Markey BK, Leonard FC, FitzPatrick ES, Fanning S and Hartigan PJ, 2011. *Veterinary microbiology and microbial disease*. 2nd Edi. Wiley-Blackwell.
- Schlafer DH and Gifford AT, 2008. Cystic endometrial hyperplasia, pseudo-placentational endometrial hyperplasia, and other cystic conditions of the canine and feline uterus. *Theriogenology* 70:349-58.
- Senturk LM and Erel CT, 2008. Thin endometrium in assisted reproductive technology. *Curr Opin Obstet Gynecol* 20:221-8.
- Smith FO, 2006. Canine pyometra. *Theriogenology* 66:610-2.
- Soldera J, Nedel WL, Cardoso PR et al., 2013. Bacteremia due to *Staphylococcus cohnii* ssp. *urealyticus* caused by infected pressure ulcer: case report and review of the literature. *Sao Paulo Med J* 131:59-61.
- Suvarna SK, Layton C and Bancroft JD, 2019. *Bancroft's Theory and Practice of Histological Techniques E-Book* (Elsevier Health Sciences).
- Verstegen JG and Verstegen-Onclin K, 2008. Mucometra, cystic endometrial hyperplasia, and pyometra in the bitch: advances in treatment and assessment of future reproductive success. *Theriogenology* 70:364-74.
- Winn Washington C and Elmer Koneman W, 2006. *Koneman's color atlas and textbook of diagnostic microbiology* (Lippincott Williams & Wilkins: Philadelphia).
- Zanghi A, Catone G, Marino G, et al., 2007. Endometrial polypoid adenomyomatosis in a bitch with ovarian granulosa cell tumour and pyometra. *J Comp Pathol* 136:83-6.
- Zhang DJ, Li G, Xu R, et al., 2015. Follicle-stimulating hormone promotes age-related endometrial atrophy through cross-talk with transforming growth factor beta signal transduction pathway. *Aging Cell* 14:284-7.