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Microbiological and Haemato-biochemical Studies in Canine Pyometra

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ABSTRACT

Background: Canine pyometra, is a pathological condition that results in the accumulation of purulent exudate in the uterus due to hormonal imbalance in female dogs. This is the most prevalent hormonally mediated reproductive disorder and life threatening disease of intact female dogs. The objective of the present study was to assess the physiological, haematological and biochemical changes and isolate the pathogenic microorganism present in the uterus of pyometra bitches.

Methods: This study was carried out in ten bitches with a history of lethargy, depression, in appetence, polydipsia, occasional vomiting and vaginal discharge presented for treatment at the Veterinary Poly Clinic, Coimbatore, Tamil Nadu. The disease was diagnosed by history, clinical signs, physical examination, imaging techniques haematology and biochemical study. The involvement of the pathogenic organism was confirmed by microbial examination of the pus-filled uterus after the ovariohysterectomy.

Result: The result showed that physiological parameters rectal temperature, heart rate and respiration rate were increased in pyometra affected dog than healthy one. The haematological parameters of red blood cell count, haemoglobin and packed cell volume were decreased in pyometra affected dogs than healthy dogs. Leukocytosis, neutrophilia, lymphocytosis, monocytosis and eosinophilia condition also observed in pyometra affected dogs. Biochemically, increased Blood urea nitrogen (BUN) creatinine and hyperprotenimia were observed in pyometra dogs. Increased activity of hepatic enzyme Alanine transaminase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) were also observed in pyometra dogs. The major pathogenic microorganisms involved in pyometra are *Escherichia coli* (40.25%), *Staphylococcus* (20.46%), *Streptococcus* (20.32%), *Pseudomonas* (6.41%), *Proteus* (6.32%) and *Pasteurella* (6.24%). Thus the dogs with pyometra show significant changes in physiological, haematological, biochemical parameters and growth of pathogenic microorganism in uterine fluid were the important indications of pyometra affected dogs.

Key words: Canine pyometra, Cystic endometrial hyperplasia, Haematology, Microbiology.

INTRODUCTION

Canine pyometra, is the most prevalent reproductive disease in bitches, impacting up to 25% of unspayed female dogs, particularly during the diestrus phase of the oestrus cycle and progesterone dominant uterus. It is a hormonally mediated reproductive disorder of middle to old aged intact females characterized by cystic endometrial hyperplasia (CEH) followed by secondary bacterial infection. It is the chronic sub-acute metritis or endometritis in bitches characterized by the proliferation of endometrial glands resulting in the formation of fluid-filled cysts and luminal uterine contents. It is commonly affected in all age group of intact female but most preferably affected in aged dogs before they reach ten years of age. Pyometra is more common in middle-aged and older dogs and it is thought to be related to repeated estrous cycles. It is a reproductive disorder characterized by a pus-filled uterus that developed mainly as a result of hormonal and physiological changes. The internal environment of the uterus contributes to the growth of an opportunistic pathogens, leading to a potentially fatal infection. This is a very serious life threatening pathological condition in bitches. It occurs in older or middle-aged, intact bitches after one or more non-pregnant estrous cycles. It occupies a leading position among uteropathies found in the aged female dogs.

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Pyometra in the bitch is a hormonally mediated disease. Prolonged or recurrent progesterone stimulation of the endometrium results in cystic endometrial hyperplasia and bacterial infection occurring at that time; causes pyometra in dogs. Increased progesterone concentrations (>40 ng/ml) following ovulation encourage the growth of the endometrium (endometrial hyperplasia) and glandular secretion causes an accumulation of uterine secretions, which makes excellent media for the growth of bacteria (Bigliardi *et al*., 2004).

Pyometra is seen in female dogs in the diestrum phase of estrus cycle or pseudo-pregnancy condition.

The diestrum lasts 60-90 days in a bitch. During diestrum, progesterone hormone stimulates, endometrial proliferation and accumulation of glandular secretion which is as an excellent medium for growth of bacterial in the uterus. Further, diestrum inhibits local leukocyte responses and uterine resistance to bacterial infection. These effects aggregate during multiple estrous cycles (Sachan *et al*., 2019). Pseudopregnancy or pseudocyesis, or a phantom pregnancy in dogs is a common condition which can occur in around 80% of unspayed female dogs. It occurs when progesterone levels, which rise after ovulation, begin to fall. Decreasing progesterone leads to an increase of the hormone prolactin. These changes that occur to the uterus during pseudopregnancy increase the risk of pyometra in dogs.

Canine pyometra is classified based on the status of the cervix as open-cervix or closed-cervix; however, the closed type is a more serious condition that requires surgical intervention to prevent concurrent sepsis and mortality (Smith, 2006). The pathogenesis of pyometra is unclear, but involves both hormonal and bacterial factors. The hormone progesterone level increase in luteal phase of estrus cycle causes endometrial proliferation, which will stimulate endometrial secretion, inhibit myometrial contractions, cause cervical closure and have negative effects on uterine immunity while protecting against infections (Sant' Anna *et al*., 2014). In dog's affected with pyometra, the disease causes bacteremia, endotoxemia and systemic inflammation affecting organs such as the heart, liver, kidney and bone marrow, raising concerns about its possible role in sudden death in affected dogs. The most characteristic alteration is an inflammatory leukogram with a marked elevation of the total white blood cell count. The objective of the study was to assess the changes of physiological, hematological and biochemical parameters and involvement of pathogenic microorganisms in pyometra affected bitches.

MATERIALS AND METHODS

The present study was carried out at Department of Veterinary and Animal Sciences, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu during April 2023 to June 2024. Ten clinical cases of different breeds in age group of four to nine years were brought to Veterinary Poly clinic, Coimbatore with known history of lethargy, depression, anorexia, polydipsia, polyuria, occasional vomiting, abdominal distension and purulent vaginal discharge were selected for this study. Further, ten healthy non-pregnant bitches presented for routine ovario hysterectomy were also considered for comparison of physiological, hematological and biochemical attributes. The presumptive clinical diagnosis was based on the case history, clinical signs and abdominal palpation. The diagnosis was confirmed by a macroscopic examination of a pus-filled uterus during and after the ovario hysterectomy. The imaging techniques radiography and ultrasonography also used for accurate diagnosis of canine pyometra case. Radiography of abdomen of bitches were taken using a 200 mAs capacity X-ray machine. B-mode ultrasonography was used to visualize the uterus using the Esaotemy Lab 40 model with the trans-abdominal (convex) probe at 5.0 MHz and the uterine horn diameter was measured using the machine's inbuilt digital caliper.

Blood samples were collected for haematological and biochemical studies during clinical examination of all bitches. Blood was collected from cephalic vein after an ultrasonography examination. In hematological studies, 1 ml of collected blood was poured into a sterile vial containing the anticoagulant ethylene diamine tetra acetic acid (EDTA-2 mg/ml). In biochemical study, remaining blood was transferred to a test tube and allowed to be centrifuged to separate serum samples for analysis. The hematological parameters were determined by using an automated blood counter (MS4E, French).

The biochemical parameters of blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total protein were determined by commercially available assay kits with the help of a biochemical analyzer (Fuji Dry Chem NX500 Analyzer, Clinical Chemistry Analyzer, India). After ovario hysterectomy, the contents of the uterus were aseptically aspirated by a sterile syringe. Then the samples were sent to the microbiology laboratory. The cultures were grown on 5% sheep blood agar (Himedia®, Mumbai, India) and the plates were incubated aerobically at 37°C for 72 hours. The isolated microorganisms were analyzed by Gram staining, catalase, coagulase and esculin tests with a 6.5% NaCl tolerance (Holt *et al*., 1994). Gram-negative bacteria were placed on Mac Conkey agar and identified by the Bactray system (Laborclin® Pinhais, Brazil). The data obtained from both pyometra and healthy bitches subjected to statistical analysis of the hemato-biochemical parameters of healthy and pyometra bitches were compared using a paired t-test.

RESULTS AND DISCUSSION

Clinical signs

The history of lethargy, depression, anorexia, polydipsia, occasional vomiting and purulent vaginal discharge seen in all the bitches selected for this study. Based on the history of intact bitches having vaginal discharge combined with polyuria and polydipsia, abdominal distension and palpable enlarged uterus were suspected case of pyometra. The pyometra bitch showed various clinical signs that helped in the diagnosis of pyometra. Clinical signs such as lethargy/depression, anorexia, brown chocolate malodorous vaginal discharge, polydipsia, polyuria, tachypnea, abnormal mucous membranes, vomiting, fever, dehydration and palpable uterine distension were observed in selected bitches and percentage of clinical signs in pyometra affected bitches are shown in Table 1.

The brown chocolate malodorous vaginal discharge with discolouration of the perivulval tissue observed in 58.33% of dogs suspected of pyometra case. According to Feldman and Nelson (2004) study indicate that systemic signs such as depression, listlessness, lethargy, anorexia, vomiting and weight loss were observed pyometra dogs. Fransson and Ragle (2003) reported that pyometra affected bitches were dehydrated and hypothermic. Hyperthermia is supposed to be associated with uterine inflammation and secondary bacterial infection, as well as septicaemia or bacteraemia.

Radiography and ultrasonography

Radiography and ultrasonography are the two important techniques used for diagnosing pyometra bitches. All the selected dogs were confirmed to have pyometra by radiography and ultrasonography techniques. Abdominal radiography left lateral and ventro dorsal views were taken in bitches. The lateral radiograph showed two gas-filled tubular structures, measuring up to 3.5 times the height of the body of the 5th lumbar vertebra and there was a cranio dorsal displacement of the small bowel and a twisted, uniform tubular opacity in the caudo-ventral abdomen. The ventro dorsal radiograph showed that the two gas-filled structures were parts of the same, slightly contracted, tubular structure. In the caudal and mid abdomen the tubular structure was medial to the descending colon and had a soft tissue/fluid opacity in this region. The tubular structure then turned to the right crossing the midline at the level of the two first lumbar vertebrae. Following the radiographic examination, abdominal ultrasonography was performed to observe the distention of the uterus with presence of multiple anechoic sac with pus accumulation and endometrial hyperplasia. Uterine horns were enlarged and filled with infectious fluid and gas. The right horn was up to 4.8 cm in diameter, thin-walled, distended and fluctuant due to the gaseous and liquid content. The left horn was 3.6 cm in diameter and contained fluid and gas. The right medial iliac lymph node was mildly hypoechoic and rounded compared to the left one. Free fluid and free gas were not found in the abdomen.

Radiology of the abdomen confirmed the uterine enlargement in the bitches. The x-rays identified a uterus that emerges from the pelvis as dilated and showed a homogeneous and sacculiform structure with the dorsal and cranial displacement of the small intestine. The radiography is used as an aid in diagnosing pyometra in the bitches, but it is generally inconclusive. In the early stages of disease progression, the uterine horns classically emerge distended with hypoechoic to hyperechoic fluid, with or without flocculation. Thickened irregular boundaries of the uterine wall and small hypoechoic areas steady with cystic proliferation of endometrial glands were observed. The amount of uterine pus depends on the patency of the cervical lumen. In close cervix pyometra, there is a larger amount of uterine pus present in the lumen. Lee *et al*. (2016) reported enormous pus in the uterus lumen in a pyometra condition. Canine pyometra is the condition considered as a potential medical emergency, though it is often difficult to diagnose as there may be mild clinical signs and laboratory changes (Klainbart *et al*., 2017). The disease has been progressing for a longer time since it was diagnosed, which may have led to a more severe illness (Jitpean *et al*. 2017).

Haematological and biochemical parameters

The detail values of physiological parameters *viz*. rectal temperature, heart rate, respiration rate and haematological parameters, *viz*., haemoglobin, red blood cell count, packed cell volume, total leukocyte count and serum biochemical parameters, *viz*., blood urea nitrogen creatinine alanine transaminase, aspartate amino transferase alkaline phosphatase and total protein values are represented in Table 2.

The physiological parameters, *viz*. rectal temperature, heart rate and respiration rate of the pyometra-affected bitch, were significantly (P<0.01) higher (102.70±0.23°F, 140.62±2.41 beats/min and 38.58±1.45 per min, respectively) in comparison to the healthy bitch (100.10±0.24°F, 121.08±2.32 beats/min and 27.25±1.20 per min, respectively). In the present study packed cell volume percentage was decreased significantly (P<0.01) in the bitches affected with pyometra indicating a mild normocytic, normochromic and regenerative type of anaemia.

The haematological parameters, *viz*., Haemoglobin, packed cell volume and red blood cell count were significantly (P<0.01) decreased in pyometra affected bitches in comparison to healthy controls, which indicated anaemia. Total leukocyte count showed a highly significant (P<0.01) elevation in pyometra dogs than healthy dogs, reflecting the higher amount of inflammatory response initiated by the disease and due to diffused suppurative inflammation of the uterus to combat the infection (Nath *et al*., 2009).

The differential leucocyte count (DLC) analysis revealed neutrophils, lymphocyte, monocyte and eosinophil

Table 1: Clinical signs of pyometra bitches.

Clinical signs	Percentage (%)
Lethargy/Depression	100.00
Anorexia	100.00
Vaginal discharge	58.33
Polydipsia	41.66
Polyuria	50.00
Tachypnea	41.66
Abnormal mucous membrane	75.00
Vomiting	16.66
Fever	66.33
Dehydration	75.00
Palpable uterine distension	100.00

count in both healthy and pyometra affected bitches. Neutrophilia, lymphocytosis, monocytosis and eosinophilia condition observed in the pyometra affected bitches. Leukocytosis in dogs with pyometra infection may be caused severity of the inflammation varying between animals. These findings are in concordance with the earlier reports (Dabhi *et al*., 2009; Jena *et al.,* 2013; Babu *et al*., 2018).

Neutrophillia with a regenerative shift to the left might be due to stagnation of purulent exudate in the uterus, causing a chemotactic effect on neutrophils, leading to increased granulation rate and lymphopenia might be due to severe stress and high monocytes might be due to the chronic nature of the suppurative process. Neutrophilia are a typical hematological feature of bitches with pyometra, possibly due to the effects of intrauterine toxins (Singh *et al*., 2006; Nath *et al*., 2009). Neutrophils, lymphopenia, eosinophilia and monocytosis were common in bitches with pyometra. Gupta and Dhami (2013) reported that a reduced haemoglobin, red blood cell count, packed cell volume level of and platelets, along with an elevated level of erythrocyte sedimentation rate (ESR), total leucocyte count (TLC) and polymorpho nuclear leucocytes (PMN) cells, indicates toxaemia, whereas a raised level of leucocytes, polymorphonuclear leukocytes and declining lymphocytes point out recovery from toxaemia. Therefore, these altered haematological values can be used to aid in the diagnosis and prognosis of canine pyometra.

The biochemical parameters, *viz*., total protein, blood urea nitrogen creatinine, Alanine Transaminase, Aspartate Aminotransferase Alkaline Phosphatase were significantly ($P<0.01$) increased in pyometra-infected bitches as compared to healthy ones. In the present study out of 10 bitches six bitches were diagnosed with open cervix pyometra. According to Jitpean *et al*. (2014) the open cervix is more common than closed cervix pyometra in dogs. The present findings indicate that bitches with closed cervix pyometra are in a more serious state than those with open cervix pyometra because in closed cervix pyometra, pus and bacterial products accumulate in the uterus leads to more severe infection.

Pyometra-affected bitches showed various abnormalities in haematological and biochemical variables. The result of the present findings, abnormal patterns of blood profiles were similar to Kaymaz *et al*. (1999) study. The anemia in pyometra bitch is attributed to toxic depression of the bone marrow and/or loss of red cells into the uterine lumen. Another reason for anaemia in the pyometra case, along with the diapedesis of erythrocytes into the uterine lumen, is that a shortened life span of circulating erythrocytes due to iron deficiency may also be responsible for the anaemia (Samantha *et al*., 2018). The accordance with the similar findings Hagman *et al*. (2009). Reported that leukocytosis condition in pyometra bitches. The noticeable leukocytosis observed in the present study is due to a bone marrow inflammatory response and diffuse inflammation of the uterine lumen to battle the infection. The neutrophilia recorded in the present investigation may be attributed to the defence mechanism of the uterus in response to the invading microorganisms (Sevelius, 1990). Increased

Table 2: Haematological and biochemical parameters of healthy and pyometra bitches.

Parameters	Healthy bitches	Pyometra bitches	t-value
Temperature (°F)	100.10±0.24	102.70±0.23	9.330**
Heart rate (bpm)	121.08±2.32	140.62±2.41	7.918**
Respiration (per minute)	27.25±1.20	38.58±1.45	6.175**
Haemoglobin (gm/dl)	11.54±0.34	8.16±0.40	6.360**
Red blood cell - RBC ($\times 10^6$/µL)	7.60±0.43	6.37±0.35	3.600**
Packed cell volume PCV (%)	42.70±1.18	31.70±0.96	6.917**
Leukocyte count - WBC ($\times$ 1000/µL)	12.65±5.98	34.26±8.97	5.343**
Neutrophil (%)	50.33±3.73	88.25±2.78	12.390**
Lymphocyte (%)	38.00±1.92	57.29±4.00	4.511**
Monocyte (%)	4.47±0.48	6.87±0.34	5.701**
Eosinophil (%)	1.90±0.24	2.86±0.36	3.763**
Mean corpuscular volume (fL)	53.12±1.60	54.50±2.00	0.582
Mean corpuscular haemoglobin (pg)	19.95 ±0.47	14.58±0.88	5.731**
Mean corpuscular haemoglobin concentration (%)	29.91±0.37	23.12±1.11	5.422**
BUN-Blood urea nitrogen (mg/dl)	28.12±2.01	68.25±6.39	7.175**
Creatinine (mg/dl)	1.68±0.22	2.40±0.29	5.532**
ALT- Alanine transaminase (IU/L)	45.04±6.03	59.64±7.55	3.838**
AST- Aspartate aminotransferase (IU/L)	23.00±2.08	52.75±3.35	6.210**
ALP- Alkaline phosphatase (IU/L)	46.79±4.36	98.64±4.71	8.948**
Serum protein (g/dl)	7.41±0.30	9.56±0.67	3.260**

The value given in each cell is the mean of ten observations.

Values are mentioned as Means±SE.

**Significant at $P<0.01$.

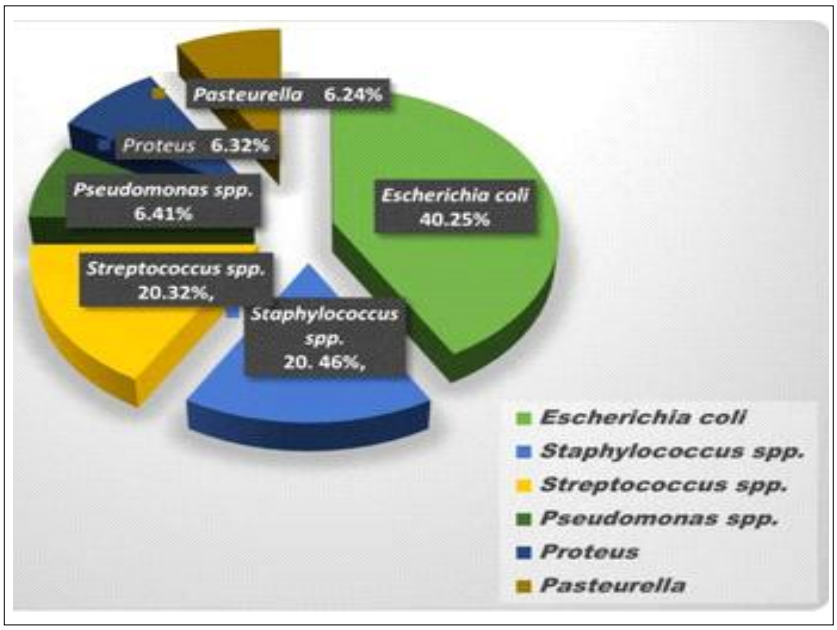


Fig 1: Microorganism isolated from the uterus in pyometra affected bitches.

concentrations of blood urea, nitrogen and creatinine were common complications of pyometra and they may also be caused by dehydration associated with anorexia and vomiting (Hagman *et al*., 2014).

Chojong and Kimhyesod (2000) and Sharma (2004) reported that high serum creatinine concentrations were observed in bitch with pyometra. The destruction of tissue due to the effect of endotoxin can be attributed to elevated levels of serum urea nitrogen and creatinine. The destruction of tissue due to the effect of endotoxin can be attributed to elevated levels of serum urea nitrogen and creatinine (Mohan *et al*., 2015). Gayakwad *et al*. (1999) observed that increased blood urea nitrogen in pyometric bitches indicate the hampered efficiency of the kidneys to remove nitrogenous waste from the circulation. The elevated creatinine level in blood might be due to massive destruction of tissue elements coupled with urinary insufficiency indicating renal failure (Sahoo *et al*., 2012).

The liver has a significant role in metabolism, digestion, detoxification and the elimination of substances from the body. The liver function tests typically include alanine transaminase (ALT) and aspartate transaminase (AST), alkaline phosphatase (ALP) and total protein. The elevated level of hepatic enzyme AST and ALT, indicated that hepatocellular damage and ALP indicated cholestasis in pyometra affected bitches. Jitpean *et al*. (2014) reported that bitch affected in pyometra increased blood urea nitrogen, creatinine, ALT, AST, ALP and globulin; however, albumin level highly significantly decreased, thus causing an enhanced ratio of globulin: albumin in pyometra cases.

Microbiology

The pathogenic microorganisms isolated from the uterus of experimental bitches were *Escherichia coli, Staphylococcus, Streptococcus, Pseudomonas, Proteus* and *Pasteurella*. The coliform bacteria, *i.e*., *Escherichia coli*, was the predominant pathogen (40.25%) among other pathogenic organisms isolated from the uterus (Fig 1).

Weiss *et al*. (2004) and Emanuelli *et al*. (2012) found similar diversity in the etiological agents of pyometra. Wadas *et al*. (1996) found the infection of the uterus occurred *via* an ascent of faecal bacteria through the vagina during the oestrus when the cervix is relaxed. Ruthrauff *et al*. (2009) reported that, apart from bacterial virulence, some other factors are associated with pyometra in the bitch, such as an inadequate innate immune response and hereditary predisposition.

CONCLUSION

Pyometra is mediated by progesterone and aggravated by estrogens (repeated cycles of exposure to estrogen and progesterone; estrogen exposes the endometrium, which increases progesterone receptors). There was significant alteration in haematological and biochemical parameters in pyometra affected bitches compared to healthy bitches. The most common pathogenic bacteria isolated from the uterus of pyometra dogs are *Escherichia coli* and followed by other bacterial organisms in uterus are *Staphylococcus spp*., *Streptococcus spp*., *Pseudomonas spp*. *Proteus* and *Pasteurella*. These observations may be explained by the fact that dogs with closed cervix pyometra may have experienced a longer duration of disease progression prior to diagnosis and treatment, leading to a more severe illness.

Conflict of interest

The author declare no conflict of interest regarding publication of this article.

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