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Comparative Hemato-biochemical Evaluations of Canine Pyometra

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ABSTRACT: Canine pyometra results in the accumulation of pus in the progesterone-influenced uterus of unmated canines. Clinical symptoms like purulent vaginal discharge, abdominal pain, lethargy, depression, anorexia, polyuria, polydipsia and vomiting are observed. Without timely treatment, it can progress to peritonitis, sepsis, nephritis, and the dysfunction of multiple organs. Systemic effects of canine pyometra can be indicated by various hemato-biochemical parameters. Sixteen female dogs with open and closed pyometra of various breeds, ages ranging between 2-12 years, were examined on clinical aspect at City Teaching Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Anjora, Durg, Chhattisgarh. The suspected diagnosis was based on history taken from owners and overt clinical signs which were later confirmed by transabdominal ultrasonographic examinations. Analysis of Haemato-biochemical parameters before starting the treatment protocols in all the pyometra-affected dogs was performed. Approximately, three milliliters of blood were collected from cephalic and saphenous veins aseptically in EDTA-containing vials (TLC and Hb) and clot-activated vials (BUN and Creatinine) for hemato-biochemical evaluation. An increase in the number of leucocytes and reduced hemoglobin concentration were found in canine pyometra. Increased creatinine and BUN values show compromised efficiency of kidneys in filtering nitrogenous wastes from the blood circulation in pyometra-affected dogs.

Keywords: Haemoglobin, BUN, White blood cell count, Canines, Pyometra, Creatinine.

INTRODUCTION

Canine pyometra is an infectious condition that affects the uterus of unmated dogs and is known to trigger systemic inflammatory reactions that result in various systemic dysfunctions (Sethi *et al.*, 2019). It is among the most frequent reasons why older animals die in a short course of time. Clinical signs and symptoms include vomiting, lethargy, inactive, anorexia, polyuria, polydipsia, and purulent vaginal discharge (Jitpean *et al*., 2014). Without timely treatment, it can progress to peritonitis, sepsis, nephritis and the dysfunction of multiple organs (Hagman, 2022). Systemic effects of canine pyometra can be indicated by various hemato-biochemical parameters. Pyometra, resulting from bacterial infection and endogenous or exogenous steroid hormones like, estrogen or progesterone (Sureshkumar *et al*., 2023). Bacterial development in the uterus and the ensuing immune reaction impact the dog systemically in the majority of pyometra cases. *Escherichia coli* is the most often isolated bacterium from the uterus (*E. coli*). *E. coli* has the same potential to produce endotoxins during periods of rapid development or when they die as other Gram-negative bacteria. Many of the symptoms linked to pyometra are thought to be caused by endotoxemia. In addition to *E. coli*, other bacteria that have been recovered from the infected uterus include Klebsiella organisms, streptococci, staphylococci, and anaerobic bacteria called pseudomonads. Research has demonstrated that progesterone increases the release of endometrial glands and inhibits uterine contractions, so fostering an intrauterine environment that is conducive to the development of germs. Early studies raised suspicions about the significance of hormone effect on the uterus in the development of pyometra based on based on the observation that the disease most commonly occurs during progesterone influence in diestrus.

There are two forms of pyometra: closed cervix pyometra and open cervix pyometra. A bacterial uterine infection combined with cervix dilatation (therefore the cervix is "open") characterizes open-cervix pyometra. The purulent discharge from the uterus can be drained through the dilated cervix. A foul-smelling, sanguineous mucopurulent vaginal discharge from the diseased bitch is another symptom. Affected bitches may not exhibit any clinical symptoms other than

vaginal discharge; bitches with open cervix pyometra are often less systemically unwell than bitches with closed cervix pyometra especially early in the course of the disease. Throughout all of the open cervix pyometra patients in the current research, vomiting, polyuria, polydipsia, diarrhea, and severe abdominal distension were consistent symptoms. But these clinical indications were noticeably less than in situations of the closed cervix when there was very little vaginal discharge. When the cervix is closed and the infection is trapped inside the uterus with no way out, it is known as closed-cervix pyometra. This essentially causes an internal abscess inside the abdominal cavity with potentially lethal consequences.

The expression of the systemic response to an infectious or non-infectious condition linked to a significant release of inflammatory mediators is known as systemic inflammatory response syndrome or SIRS. According to Yazlik *et al.* (2023), there are previously published criteria for diagnosing SIRS in dogs, which include abnormalities in leukocyte count, heart and respiration rate, and body temperature. Several laboratory markers represent the systemic consequences of pyometra. An inflammatory leukogram with a noticeable increase in the total white blood cell count is the most distinctive change. Kidney disease is indicated by biochemical indicators such as plasma creatinine and blood urea nitrogen (Shah *et al.*, 2017).

Although the etiopathology of pyometra remains poorly understood despite decades of research, there is a connection between this issue and compromised immune function, particularly decreased lymphocyte activity (Yazlik *et al.*, 2023). The goal of this study was to examine changes in a few key blood biochemical and hematological parameters of pyometra-infected dogs to make recommendations for the early diagnosis and care of companion and breeding dogs.

MATERIAL AND METHODS

A. Source of study

Sixteen female dogs with open and closed pyometra of various breeds, ages ranging between 2-12 years, were examined on clinical aspect at City Teaching Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Dau Shri Vasudev Chandrakar Kamdhenu University, Anjora, Durg, Chhattisgarh. The suspected diagnosis was based on history taken from owners and overt clinical signs which were later confirmed by transabdominal ultrasonographic examinations. Analysis of Haemato-biochemical parameters before starting the treatment protocols in all the pyometra-affected dogs was performed.

B. Hemato-biochemical evaluation

Approximately, three milliliters of blood were collected from cephalic and saphenous veins aseptically in EDTA-containing tubes and clot-activated tubes for hemato-biochemical evaluation. The EDTA tubes were used for evaluating Total Leukocyte Cell count (TLC) and Hemoglobin (Hb) concentration. The serum extracted from the clot-activated tubes was for evaluating Blood urea nitrogen (BUN) and Creatinine concentrations. For hematological evaluation, hemoglobin concentration and total leukocyte count were estimated using Vet MS39 semiautomatic Haematological analyzer of Melet Schloesing Laboratories of Sussi France. For biochemical evaluation, Blood urea nitrogen and creatinine were estimated by using DiaSIL-100 semiautomated Biochemistry Analyzer (Systronics India Limited) as per the standard procedures given in commercial kits supplied by ARKRAY Healthcare Pvt. Ltd.

C. Treatment protocols

Sixteen pyometra-affected dogs were grouped into 4 groups according to various treatment protocols followed after hemato-biochemical evaluation presented in Table 1.

Table 1: Treatment protocols for different groups.

Groups	Treatment protocols	Remarks
Group I	Parenteral Antibiotic + Intrauterine flushing	Ceftiofur sodium was used as a parenteral antibiotic @ 2.2-4.4 mg/kg B.wt. subcutaneously for 5-7 days, for Intrauterine flushing (20ml), Gentamicin (5ml) along with Metronidazole (15ml) used for 3 days alternatively.
Group II	Parenteral Antibiotic + Intrauterine flushing + $PGF_{2\alpha}$	Inj. $PGF_{2\alpha}$ (Cloprostenol 2.5 µg/kg B.wt., Intramuscularly for 3 days continuously since the start of treatment., Parenteral Antibiotic and Intrauterine flushing similar to group I
Group III	Parenteral Antibiotic + Intrauterine flushing + Misoprostol	Tab. Misoprostol (200 µg) painted intravaginally for 3 days continuously since the start of treatment, Parenteral Antibiotic and Intrauterine flushing similar to group I
Group IV	Parenteral Antibiotic + Intrauterine flushing + Misoprostol + $PGF_{2\alpha}$	Parenteral Antibiotic and Intrauterine flushing is similar to group I, Misoprostol similar to group III and $PGF_{2\alpha}$ similar to group II.

D. Statistical Analysis

The data were statistically analyzed using SPSS software. One-way ANOVA and least significance differences (LSD) post hoc tests were used to assess the significant variation among means where $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

A. Hematological evaluations

The reference values for hemoglobin concentration are considered as 12-18 g/dl in canines. The mean hemoglobin concentration in all the treatment groups of pyometra-affected canines was less than the normal physiological range indicating anemia with mild severity. The hemoglobin concentrations varied non significantly in different treatment protocol groups. The reference values for white blood cell count are considered as 6-17 × 10^3/µl in canines. All the treatment groups had having increased white blood cell count indicating moderate to intense severity of infection. The WBC count varied significantly between various treatment groups of affected canines.

Table 2: The hematology of the pyometra-affected canines in different treatment groups.

Sr. No.	Haemoglobin (g/dl)		WBC (10^3)	
	Mean±SE	Observation range	Mean±SE	Observation range
Group I	10.50 ±0.887[a]	9.1-13.1	17.47±2.350[a]	10.9-22
Group II	11.42 ± 1.250[a]	8-14	36.25 ± 4.956[b]	23-46
Group III	10.37 ± 0.943[a]	8.5-13	27.250 ± 5.11[a]	19-40.7
Group IV	11.17 ± 0.327[a]	10.4- 12	33.60± 4.189[b]	23.9-43

Superscripts bearing different letters (a,b) differ significantly ($P<0.05$)

Regardless of the type of pyometra or age group, the anemia that is present in the majority of cases may be caused by either the chronic process of RBC migration into the uterus through diapedesis or bone marrow suppression brought on by endotoxins from systemic bacterial proliferation, which also results in leukocytosis (Feldman and Nelson 2004). When nonregenerative anemia coexists with azotemia due to renal failure, the animals' decreased erythropoietin production exacerbates their anemia. In line with the findings of Hagman *et al.* (2009); Nath *et al.* (2009); Jena *et al.* (2013); Samantha *et al.* (2018), the mean hemoglobin level was lowered in bitches afflicted with pyometra, indicating anemia. In addition to decreased feed intake and reduced erythropoiesis under toxemic conditions in severely afflicted instances reported by Dabhi *et al.* (2009); Jena *et al.* (2013), this might be caused by red blood cell loss through diapedesis into the uterine lumen.

Dogs in groups II, III, and IV which comprised those with both open and closed forms of Pyometra showed a pronounced leucocytosis. Leucocytosis increased as a result of pyometra, a severe bacterial infection that causes the bone marrow to release more immature neutrophils into the peripheral blood to fight the infection (Shah *et al.,* 2017). Additionally, there is a decrease in blood iron levels, which are high in the bone marrow and inside macrophages as ferritin (Santos dos Anjos *et al.,* 2021). Since iron is required for the growth of germs, the body uses this process, which results in a reduction in iron's bioavailability, to inhibit the growth of bacteria.

B. Biochemical evaluations

The reference values for BUN and creatinine concentration in canines are 7-32 mg/dl and 0.5-1.4 mg/dl, respectively. There is a marked increase in creatinine concentration in group I as compared to normal reference value with no significant variation between different treatment protocol groups, while increased BUN concentration in groups I, II and IV were observed with a significant variation between different groups.

Table 3: The Biochemical evaluations of the pyometra-affected canines in different treatment groups.

Sr. No.	Creatinine (mg/dl)		Blood Urea Nitrogen (mg/dl)	
	Mean±SE	Observation range	Mean ± SE	Observation range
Group I	6.41± 3.70[a]	0.93-16.67	62.20 ± 20.904[a]	20-123.8
Group II	1.54± 0.369[a]	1.02-2.62	93.60 ± 16.337[b]	52-126
Group III	1.70±0.330[a]	1.04-2.45	29.02 ± 8.707[a]	14.5-54
Group IV	1.01±0.213[a]	0.57-1.57	35.00 ± 10.319[a]	15-57

Superscripts bearing different letters (a,b) in the same column differ significantly (P<0.05)

Serum markers of kidney function, such as urea and creatinine, may rise due to immunocomplex deposition in the glomeruli, which follows endotoxemia from an *Escherichia coli* bacterial infection (Feldman and Nelson 2004; Maddens *et al.,* 2010), Urea and creatinine levels were raised, which is similar to what was shown in this experiment and may be caused by *E. coli* in the uterine secretions (Santos dos Anjos *et al.,* 2021). According to Nath *et al.* (2009), elevated BUN and creatinine levels may be the result of toxemia, which can cause suppurative processes that lead to the breakdown of body protein. Additionally, decreased renal perfusion and dehydration can result in immune complex deposition in the glomeruli, causing glomerulonephritis and proximal tubular damage, which can lead to renal failure. However, according to Nak *et al.* (2004), pyometra may result in renal failure because of the way renal tubules are affected by

bacterial toxins, particularly those produced by Escherichia coli. According to Singh *et al.* (2006), plyometric bitches with higher BUN levels may have higher urea synthesis or improper urea clearance. Either prerenal or renal variables were linked to impaired urea excretion in urine. Pre-renal variables such as hypotension or hemoconcentration may cause the glomerular filtration rate to drop, which would reduce the kidney's efficiency.

On the other hand, it was observed by Hagman *et al.* (2006) and Hagman *et al.* (2009) that the BUN and creatinine levels of the afflicted dogs did not deviate considerably from normal levels, indicating that the majority of the dogs had normal kidney and liver function and no hepatic damage. According to Verstegen *et al.* (2008), unless pre-renal azotemia occurred as a result of dehydration, serum blood urea nitrogen and creatinine concentrations were often not raised. According to Hagman *et al.* (2009); Singh *et al.* (2006), there was no discernible difference in the creatinine levels between sick dogs and the other group.

CONCLUSIONS

Pyometra is a multifactorial disease that commonly occurs during the diestrus phase of female canines. An increase in the number of leucocytes and reduced hemoglobin concentration were found in canine pyometra. Increased creatinine and BUN values show compromised efficiency of kidneys in filtering nitrogenous wastes from the blood circulation in pyometra-affected dogs.

FUTURE SCOPE

However, very limited information on the predictive value or usefulness of platelets and their indices along with biomarkers in canines with pyometra is available. The canines that were evaluated within the scope of this study generally referred to the treatment immediately after symptoms were seen.

Conflict of Interest. None.

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