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## Haemato-biochemical studies in canine pyometra

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#### Abstract

In the present study, an attempt was made to study haematological and biochemical changes in bitches affected with Pyometra. Eight bitches with Pyometra (both open and closed type) of six different breeds, mean age 7.6 years (range 5-12 years) were clinically examined and diagnosed for Pyometra. The presumptive clinical diagnosis was based on case history, clinical signs and ultrasonography or radiography or both. The diagnosis was verified by gross examination of a pus-filled uterus during and after the ovariohysterectomy. The blood was collected in sterile test tubes from cephalic vein using 20 gauge needles with all aseptic precautions for haematological study. Total leucocyte count (TLC), differential leucocyte count (DLC), total erythrocyte count (TEC), reticulocyte count and haemoglobin (Hb) concentration were determined for haematology and as biochemical parameters blood urea nitrogen (BUN) and plasma creatinine were estimated to assess the renal dysfunction. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) total plasma protein (TP) and albumin was also determined in the pyometric bitches. Haematology of pyometra cases showed leucocytosis, neutrophilia, lymphocytopenia and normocytic and normochromic anemia. Biochemically, the increased BUN and creatinine and hyperproteinemia were observed. Alterations in the haematological and biochemical parameters were more marked in cases where the cervix was closed.

**Keywords:** Biochemical, Haematological, Canine, Hyperplasia, Pyometra

#### 1. Introduction

Pyometra is one of the common causes of death in older female dogs and kidney damage with nephritis is often associated with it (Smith, 2006; Roberts, 1999) <sup>[1, 3]</sup>. Clinical symptoms are well described and derive from the site of infection (purulent vaginal discharge, abdominal pain) and more systemic effects (lethargy, depression, anorexia, polyuria, polydipsia and vomiting) (Dabhi and Dhama, 2007) <sup>[2]</sup>. It is caused by the persistent cystic ovary and the condition is termed as cystic endometrial hyperplasia pyometra complex (CEHPC). The systemic effects of pyometra are reflected by several laboratory parameters. The most characteristic alteration is an inflammatory leukogram with marked elevation of the total white blood cell count. Biochemical parameters like blood urea nitrogen (BUN) and plasma creatinine indicate about kidney damage (Roberts, 1999) <sup>[3]</sup>. Therefore the purpose of present study was to illustrate the haematological and biochemical changes in CEHPC which are considered significant to assess the severity and type of the case. This study proves helpful in early diagnosis of Pyometra therefore the breeding life of bitch can be restored as early as possible.

#### 2. Materials and Methods

Eight bitches with pyometra (both open and closed type) of six different breeds, mean age 7.6 years (range 5-12 years), were clinically examined and diagnosed at Small Animal Clinics of the Department of Veterinary Clinical Services Complex, GADVASU, Ludhiana. The presumptive clinical diagnosis was based on case history, clinical signs and ultrasonography or radiography, or both. The diagnosis was verified by gross examination of a pus-filled uterus during and after the ovariohysterectomy. The blood was collected in sterile test tubes from cephalic vein using 20 gauge needle with all aseptic precautions. For haematological studies, whole blood was used with disodium salt of ethylene diamine tetra acetic acid as anticoagulant at the rate of 1 mg/ ml of blood. The haematological parameters were determined using automated blood counter (Beckman Coulter, Coulter diff Ac.T, USA). Total leucocyte count (TLC) was estimated manually using haemocytometer (Jain, 1986) <sup>[4]</sup>. The differential leucocyte count (DLC) was performed manually on blood smear stained by Wright-Giemsa staining method (Jain, 1986) <sup>[4]</sup>. For biochemical studies, blood containing anticoagulant was centrifuged for 10 minutes at 2500rpm.

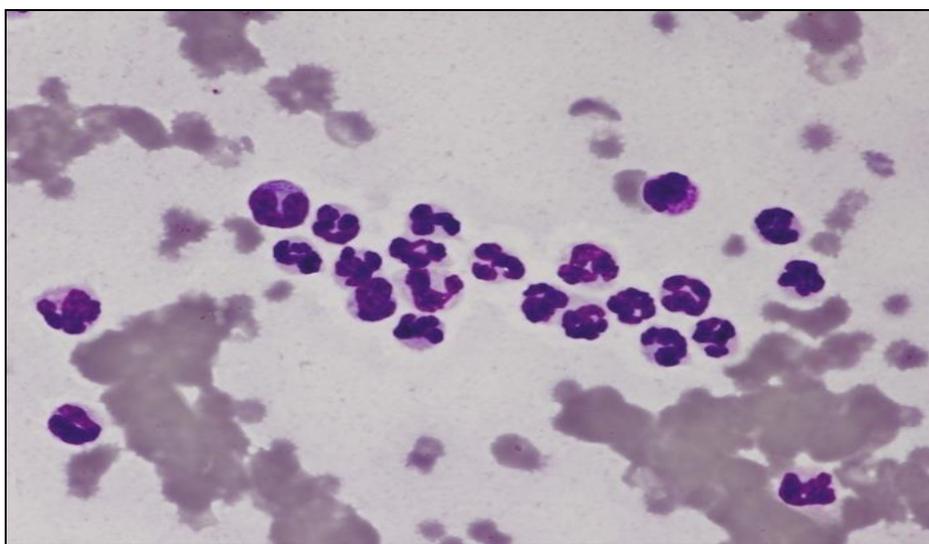
Plasma was removed and then kept in deep freeze till determination of biochemical parameters. Plasma concentration of AST, ALT, ALP, BUN, albumin, total protein (TP) and creatinine were determined by automated clinical chemistry analyzer (Vitros System Chemistry DT 60 11, Orthoclinical Diagnostics, Johnson and Johnson, USA) using standard kits (Vitros-Ortho-clinical Diagnostics, Mumbai). Reticulocyte count was done by counting reticulocytes in blood smears made from 0.5 ml of whole blood incubated with an equal volume of brilliant cresyl blue. The reticulocytes numbers were counted per 1000 RBC under oil immersion and their percentage was calculated.

**Statistical Analysis:** The arithmetical mean and standard error were calculated for all parameters using windows, Ver 5.1 Release and Stat Soft.Inc.1984-1986

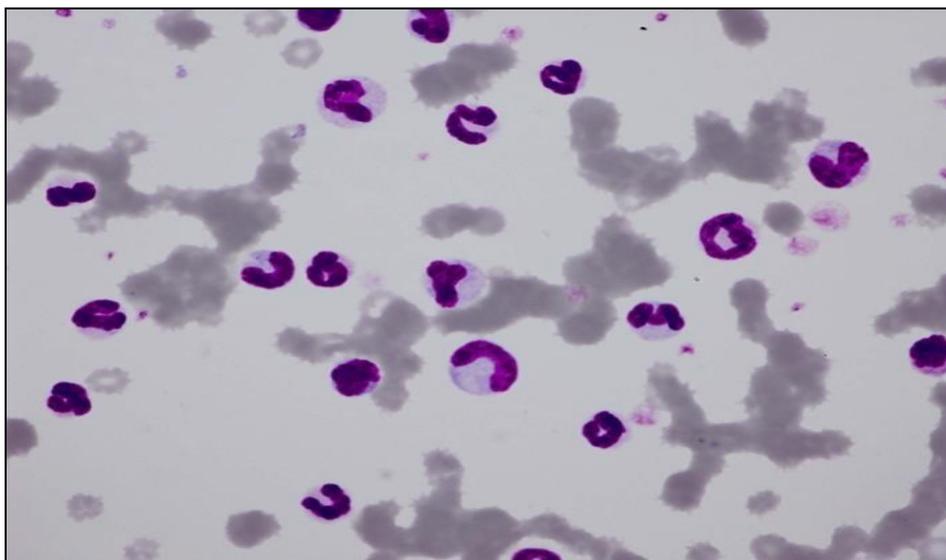
### 3. Results

#### 3.1 Hematology

The hematology (Table1) of the cases revealed leukocytosis, neutrophilia, lymphocytopenia and anemia. Marked leukocytosis ( $104.8 \pm 4.0$ ) was observed in all the pyometra cases (Fig 1: Peripheral blood smear showing marked leukocytosis; Wright-Geimsa X 1000). The leukocytosis was more marked in closed cases of pyometra complex in comparison to open cases. In present study there was marked neutrophilia ( $94.25 \pm 0.73$ ) with shift to left (Fig 2: Peripheral blood smear showing leucocytosis with marked left shift; Wright-Geimsa X 1000) and these changes were more pronounced in closed cases of CEHPC. Lymphocyte count ( $5.25 \pm 0.41$ ) was decreased in all the cases. Lymphocytopenia was more marked in the closed cases of Pyometra than open cases. All the eight cases were anaemic based on Hb ( $5.62 \pm 0.32$ ), PCV ( $18.55 \pm 1.18$ ) and TEC ( $3.24 \pm 0.59$ ).



**Fig 1:** Peripheral blood smear showing marked leukocytosis; Wright-Geimsa X 1000



**Fig 2:** Peripheral blood smear showing leucocytosis with marked left shift; Wright-Geimsa X 1000

#### 3.2 Biochemical Parameters

Blood urea nitrogen (BUN), plasma creatinine and total plasma protein (TP) (Table1) were estimated in the present study to assess renal dysfunction in the CEHPC. This study revealed significantly increased values of BUN ( $63.6 \pm 7.07$ ) and creatinine ( $2.96 \pm 0.6$ ) in CEHPC. The increase was more

marked in closed cases than in open cases. ALP ( $154.66 \pm 7.72$ ) was found to be increased whereas AST ( $55.66 \pm 1.71$ ) and ALT ( $43.66 \pm 4.68$ ) were within the normal range. TP values were significantly increased in pyometric bitches ( $9.53 \pm 0.78$ ) where as albumin was decreased.

**Table 1:** Hematological and biochemical test results in canine pyometra

Parameter	Units	Patient data		Reference range*
		Mean $\pm$ SE	Observation range	
Hb	g/dl	5.62 $\pm$ 0.32	2.0 – 8.2	12 - 18
RBC	$\times 10^6/\mu\text{l}$	3.24 $\pm$ 0.59	1.8 – 4.9	5.5 – 8.8
PCV	%	18.55 $\pm$ 1.18	8 - 25	37 - 55
MCV	Fl	60.88 $\pm$ 6.71	44.4 - 80	60 - 77
MCH	Pg	20.55 $\pm$ 1.85	11.1 – 21.6	19.5 - 24.5
MCHC	%	33.3 $\pm$ 3.37	21 – 38.4	32 - 36
WBC	$\times 10^3/\mu\text{l}$	104.8 $\pm$ 4.0	81.2 – 131.2	6 - 17
Neutrophils	%	94.25 $\pm$ 0.73	92 - 96	60-70
Lymphocytes	%	5.25 $\pm$ 0.41	4 - 6	30-40
Platelets	$\times 10^3/\mu\text{l}$	182.5 $\pm$ 17.01	100 - 250	200 - 500
Reticulocytes	%	1.29 $\pm$ 0.64	1.5 – 4.5	0.0 – 1.5
BUN	mg/dl	63.6 $\pm$ 7.07	30.4 – 110.4	7 – 32
Creatinine	mg/dl	2.96 $\pm$ 0.6	1.9 – 4.0	0.5 – 1.4
Albumin	g/dl	2.93 $\pm$ 0.03	2.9 – 3.0	3.2 – 4.2
Total protein	g/dl	9.53 $\pm$ 0.78	8 – 10.6	5.3 – 7.6
ALP	IU/L	154.66 $\pm$ 7.72	129 - 180	0 – 90
ALT	IU/L	43.66 $\pm$ 4.68	33 - 63	10 – 94
AST	IU/L	55.66 $\pm$ 1.71	51 - 63	10 - 62

#### 4. Discussion

The leucocytosis was more marked in closed cases of pyometra complex in comparison to open cases because in open cases pus drained out of the uterus through cervix but in closed cases of pyometra the pus was retained in the uterus due to closed cervix (Mojzisova *et al.*, 2000) [5]. Marked neutrophilic leukocytosis with shift to left occurs because pyometra being a severe bacterial infection stimulates bone marrow to release more number of immature neutrophils into the peripheral circulation in an attempt to combat the infection (Fransson *et al.*, 1997; Mojzisova *et al.*, 2000) [6, 5]. The anemia has been suggested to be caused by decreased erythropoiesis, so called anemia of chronic disease, and by loss of erythrocytes into the uterine lumen (Nath *et al.*, 2009) [7]. Anemia of chronic disease can be caused by a variety of disorders including chronic inflammation, in which lactoferrin and other acute phase reactants mediate an iron sequestration within the myeloid cells in the bone marrow, withdrawing iron from the normal erythropoiesis (Nelson and Couto, 1998). Non regenerative normocytic normochromic anemia has also been reported in canine pyometra by Balasubramanian *et al.*, 1993 [8].

Increased values of BUN in pyometric bitches indicate that the efficiency of kidneys to remove nitrogenous waste from the circulation is affected (Balasubramanian *et al.*, 1993 and Gayakwad *et al.*, 1999) [10, 8]. Hyperproteinemia in these cases was suggested to be due to acute phase reaction in pyometric bitches (Gayakwad *et al.*, 1999 and Singh *et al.*, 2006) [10, 12]. The elevated levels of ALP have been considered to be due to intrahepatic cholestasis (Verstegen *et al.*, 2008) [11]. Reidun *et al.*, 2007 suggested that the cause of hyperglobulinemia, concurrent with hypoalbuminemia was due to renal loss of albumin, but later studies have demonstrated only a mild to moderate urinary protein loss (Sevelius *et al.*, 1990) and interpreted the changes in serum proteins as part of an acute phase reaction (Verstegen *et al.*, 2008) [11].

#### 5. Conclusion

In this study leucocytosis was recorded along with increased neutrophils count and decreased lymphocyte count in cases of CEHPC. Anemia was evident in these cases by decreased total erythrocyte count and haemoglobin concentration. Increased values of BUN indicate that the efficiency of kidneys to remove nitrogenous wastes from the circulation is

affected in pyometric bitches. Hyperproteinemia was also an important finding in CEHPC cases which suggested acute phase reaction in pyometric bitches. Variation in all the above parameters was more pronounced in closed cases of CEHPC than in open cases probably because in closed cases pus and toxins were retained within the lumen of the uterus.

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