

Full Length Research Paper

Clinico-haematological and serum biochemical alterations in pyometra affected bitches

Amit R. Patil¹, Madhu Swamy², Astha Chandra¹ and Shobha Jawre³

¹Department of Veterinary Pathology, G. B. Pant University of Agriculture and Technology 263145, Uttarakhand, India.

²Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, JNKVV, Jabalpur. (M.P.) India.

³Department of Veterinary Surgery and Radiology, College of Veterinary Science and Animal Husbandry, JNKVV, Jabalpur. (M.P.) India.

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Canine pyometra is the infective disease of progesterone influenced uterus of intact bitches and known to cause a systemic inflammatory responses leading to multiple systemic dysfunctions. In the study twenty bitches were divided into two groups after a presumptive diagnosis of pyometra. The diagnosis was based on history, clinical examination and ultrasonography. Ten animals were placed into two groups each. Group I comprised of non-surgical cases receiving medicinal treatment and group II of the surgical cases going for ovariohysterectomy and blood samples of same animals were obtained again fifteen days after undergoing treatment either non surgical that is, medicinal (group III) or surgical (group IV), respectively. Blood was also collected from ten clinically healthy bitches. Haematological examination revealed a decrease in the total erythrocyte count (TEC), haemoglobin, and packed cell volume (PCV) in all pyometra infected animals in comparison to control value. In this study, the mean haemoglobin content of control animals was determined to be 13.42 ± 0.76 g/dl. In group I animals the mean haemoglobin was determined as 9.32 ± 2.51 g/dl, which increased after 15 days of treatment to 12.14 ± 2.14 g/dl. In pyometra infected bitches, the mean value of haemoglobin was increased 15 days post surgery to 11.07 ± 1.70 g/dl from a mean value of 7.38 ± 2.16 g/dl before surgery and moreover the mean value of TEC was increased 15 days post surgery to $5.21 \pm 1.14 \times 10^6/\mu\text{l}$ from a mean value of $3.77 \pm 1.12 \times 10^6/\mu\text{l}$ before surgery. In pyometric animals undergoing surgical treatment, the mean value of PCV before surgery was $24.18 \pm 6.85\%$, which 15 days post surgery was increased up to $36.2 \pm 4.87\%$. Band cells and Total Leucocyte count (TLC) parameters differed significantly with their control values. Leucocytosis with neutrophilia was a consistent finding along with increased number of band cells per microscopic field in pyometric cases which were decreased after both surgical and medicinal treatments. Biochemically, there were significant elevations of serum Alanine transferase (ALT), Aspartate transferase (AST), Alkaline phosphatase (ALP), Total bilirubin (BIT), Gamma glutamyl transferase (GGT), Blood urea nitrogen (BUN) and Creatinine (CRE), and decrease in protein and albumin concentration in serum of all dogs with pyometra. In pyometric animals undergoing medicinal treatment by drugs the mean value of ALT was determined as 71.86 ± 21.95 IU/L, which decreased to 58.04 ± 14.44 IU/L after treatment. In pyometric animals undergoing ovariohysterectomy, the mean value of ALT was decreased 15 days post surgery to 39.8 ± 17.91 IU/L from the mean value of 99.16 ± 44.01 IU/L before surgery. The mean value of AST was decreased 15 days post ovariohysterectomy to 21.06 ± 2.19 IU/L from a mean value of 54.02 ± 23.94 IU/L before surgery. In pyometra infected animals undergoing treatment by drugs the value of ALP was determined as 102.33 ± 46.03 IU/L, which decreased to 51.80 ± 34.16 IU/L after treatment. An increase in serum CRE and BUN values were recorded in all cases of pyometra which reduced to lower levels during both treatments in follow-ups. All the haemato-biochemical parameters were comparable to their respective reference values after either medicinal treatment or ovariohysterectomy of dogs. Thus the dogs with pyometra show significant abnormalities in haemato-biochemical parameters indicative of reversible nature of liver and kidney dysfunction in pyometra.

Key words: Canine, pyometra, clinical, haematological, biochemical alterations.

INTRODUCTION

Pyometra, by definition is the accumulation of purulent material within the uterine lumen of intact bitches, typically occurring during or immediately following a period of progesterone dominance. Progesterone has been shown to stimulate endometrial glandular secretion and to suppress contractions of the uterus, thus creating an intrauterine environment predisposed to bacterial growth. The importance of hormonal influence on the uterus in the pathogenesis of pyometra was suspected in early research, based on the observation that the disease most commonly occurs during progesterone influence in diestrus. It was later on found by De Bosschere and co-workers (2002) that the cystic and inflammatory changes of the uterine wall associated with pyometra (but not the abundant pus production) could be reproduced by injections of progesterone. In most cases of pyometra, bacterial growth in the uterus and the subsequent immune response affects the dog systemically. The most common bacteria isolated from the uterus are *Escherichia coli* (*E. coli*). Like other Gram-negative bacteria, *E. coli* may release endotoxin during vigorous growth or upon their death. Endotoxemia is believed to be responsible for many of the clinical signs associated with pyometra. Other than *E. coli*; *Klebsiella* organisms, streptococci, staphylococci, anaerobic bacteria pseudomonads have also been isolated from the infected uterus (Sandholm et al., 1975). Common clinical signs of pyometra include local sign of vaginal discharge and systemic signs such as vomiting, inappetance, polyuria/polydipsia and lethargy.

Pyometra is classified into two types: open cervix pyometra and closed cervix pyometra. Open-cervix pyometra is characterized by bacterial uterine infection with dilation of the cervix (hence the cervix is 'open'). The dilated cervix provides a route of drainage for the purulent exudate from the uterus. It also involves a malodorous sanguineous to mucopurulent vaginal discharge from the infected bitch. Renton and co-workers (1993) reports vaginal discharges from cases of open cervix pyometra. And further opined that bitches with open cervix pyometra are generally less systemically ill than bitches with closed cervix pyometra and early in the course of the disease; affected bitches may show no clinical signs other than vaginal discharge. In the present study vomiting, polyuria, polydipsia, diarrhoea and great abdominal distension were a constant feature in all the cases of open cervix pyometra. However, these clinical signs were remarkably less severe in comparison to closed cervix cases where vaginal discharge was negligible. Closed-cervix pyometra occurs when the cervix is completely closed and the infection is contained within the uterus with no route of drainage, which effectively creates an internal abscess within the abdominal cavity with the fatal sequelae.

Smith (2006) has claimed that closed cervix pyometra is a medical emergency that requires rapid intervention to prevent overwhelming sepsis and the potential of patient death. Recent findings of exaggerated toxemic clinical presentation of closed cervix pyometra are similar to his observations. Hardie and Kruse-Elliott (1990) opined that higher endotoxin levels in pyometra dogs lead to hemorrhagic diarrhoea and vomiting. Initial haemodynamic changes lead to portal hypertension, hepatosplanchnic pooling of blood and a decrease in central venous blood pressure, cardiac output and systemic blood pressure. These early changes are transitory and normal values are regained but with inadequate treatment the condition progresses to refractory hypotension. Considerable alterations in clinico-haematological and blood biochemical profiles of pyometra affected bitches have been documented by Borresen and Skrede, 1980; Stone et al., 1988 and Heiene et al., 2007. The present study was undertaken to investigate the alterations in clinical, haematological and blood biochemical profiles of dogs infected with open pyometra in order to suggest for improvement of care and management of breeding and companion bitches.

MATERIALS AND METHODS

The study was carried out in the Department of Veterinary Pathology and Disease Investigation Laboratory, Teaching Veterinary Clinical Service Complex (TVCS), College of Veterinary Science and Animal Husbandry, Jawaharlal Nehru Krishi Vishwa Vidyalyaya, Jabalpur, Madhya Pradesh (India). The investigation included a total of 20 female dogs greater than 3 years of age diagnosed tentatively with pyometra and confirmed after ultrasonography. The study population was divided into a total of 5 groups. Group I consisted of 10 clinically healthy bitches, thereby representing the control group. Naturally infected pyometra cases were divided into 4 groups as follows: Group II comprised non-surgical cases receiving medication for 15 days (pre-treatment); Group III consisted of cases receiving surgical treatment (pre-surgical); Group IV comprised of the cases receiving medicinal treatment, post-treatment cases after 15 days; and in Group V were the post-surgical cases after 15 days.

Three clinical parameters, namely rectal body temperature, respiratory rate and heart rate, were recorded in all the animals as per standard methods included in the study. A detailed history, physical and clinical examinations were taken on all suspected pyometra cases. Body temperature was measured by inserting a non-digital glass thermometer in the rectum in such a way that its bulb remained in direct contact with rectal mucosa for 2 min. The respiratory rate was measured by feeling the air movements at the nostrils. A hand was placed in front of the nostrils and the air current was felt during a period of 1 min. A stethoscope was held over the thorax in order to auscultate inspiratory and expiratory efforts of the animal. The heart rate was measured by placing a stethoscope on the chest area in between the 3rd to 6th intercostal space on left side of the animal at the level of the point of the elbow. LUB-DUB sounds audible over a period of 1 min were recorded in order to determine the heart rate. Blood samples were collected for a complete haematological and biochemical examination of relevant parameters from all cases that received a presumptive diagnosis of pyometra after their registration at TVCS. The complete blood count was performed using an automated blood

*Corresponding author. E-mail: amityvet@gmail.com.

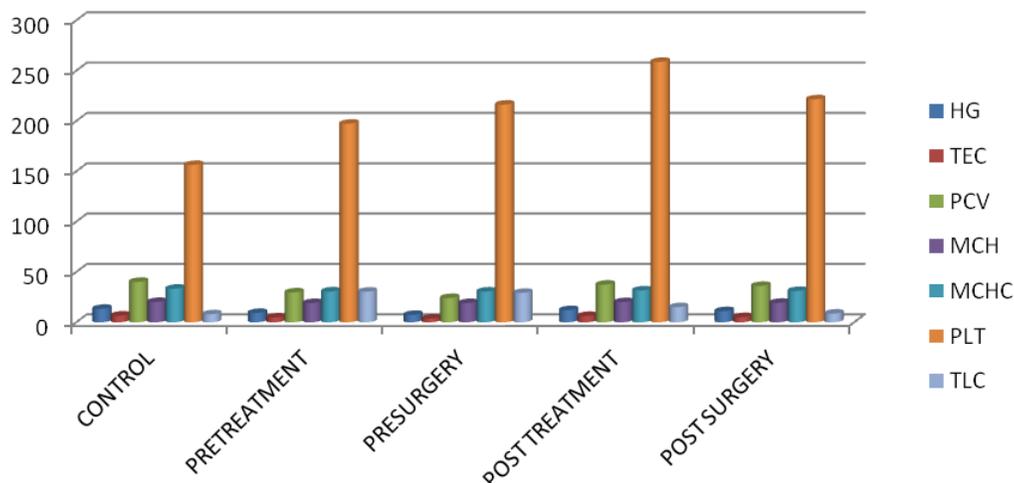


Figure 1. Means of the haematological parameters.

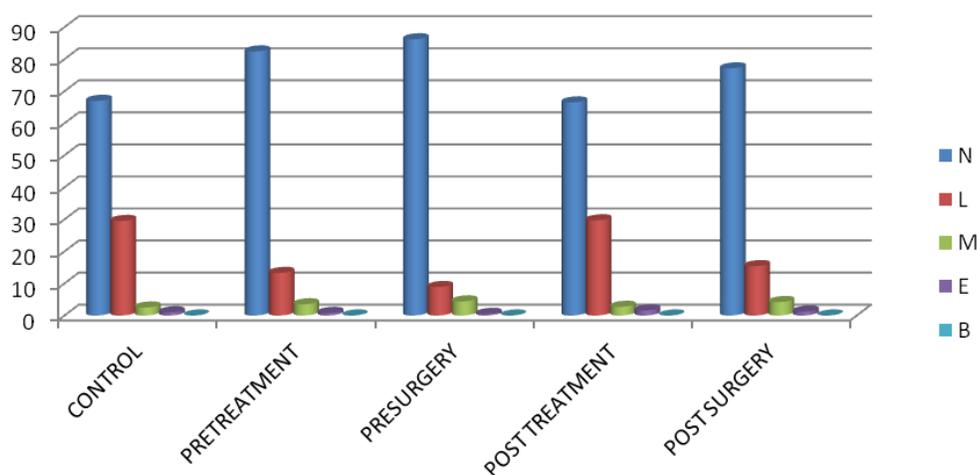


Figure 2. Means of differential leucocyte counts.

analyzer (Abacus Diatron). In addition, blood smears were prepared, and stained with Leishman's stain for determination of a differential leucocyte count and the presence of band cells.

The following biochemical parameters were measured: Alanine transferase (ALT), Aspartate transferase (AST), Alkaline phosphatase (ALP), Total plasma protein (TPP), Albumin (ALB), Total and Direct bilirubin (BIT and BID), Gamma glutamyl transferase (GGT), Creatinine (CRE), Blood urea nitrogen (BUN). The blood chemistry was performed on a Semi Auto Analyzer (Erba- CHEM-5 PLUS). The clinical, haematological and blood biochemical parameters of 10 apparently healthy bitches were also estimated as per above cited methods and compared with pyometra affected animals by using standard statistical procedures like paired t-test (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Clinical findings

All the animals affected with pyometra had a clinical syndrome consisting of lethargy, depression, inappetence/

anorexia, polyuria, polydipsia, vomiting and abdominal distension along with malodorous, sanguineous to mucopurulent vaginal discharge (Figure 5). These kinds of symptoms were reported in pyometra by Wheaton et al. (1989) and Harari (1995). The aforementioned clinical signs were absent in the control group of healthy bitches. The clinical signs were meagre with subsided levels in treated bitches after both of treatments.

Ultrasonographic examination

Ultrasonographic examination of the animals was done to confirm the diagnosis of pyometra as suggested in pyometra by Bigliardi et al. (2004). Ultrasonography revealed enlargement of both the uterine horns in the bitches clinically suspected for pyometra. Sonographically, the lumen of the horn was filled with homogenous anechoic contents with the hyperechoic bands in between which gave it fluid filled pocket like appearance (Figure 6).

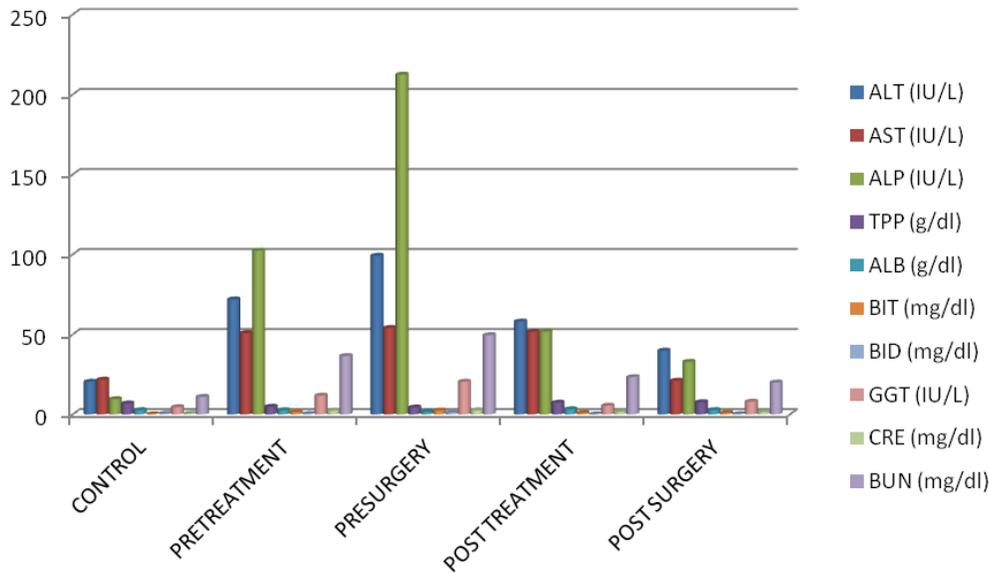


Figure 3. Means of the serum biochemical parameters.

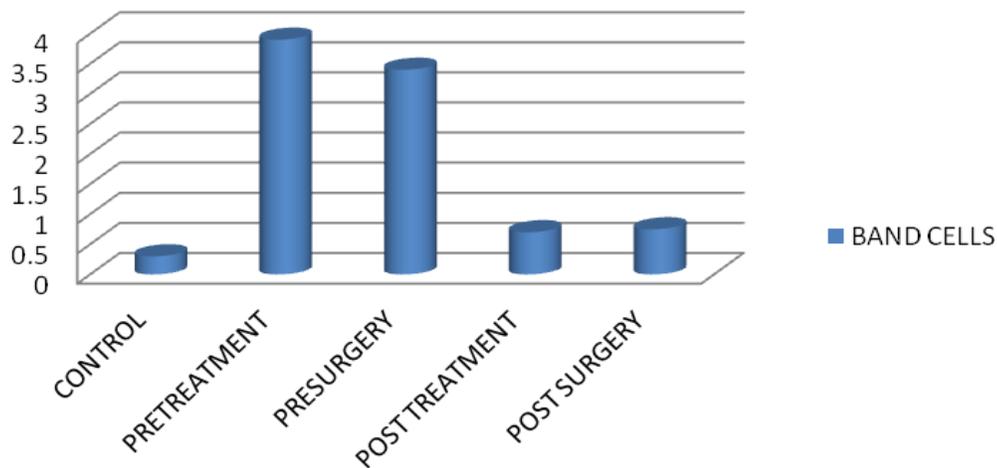


Figure 4. Means of the band cells.

Haemato-biochemical findings

The complete blood count and biochemical profile of pyometra affected bitches and healthy dogs are displayed in Tables 1 and 2. Considerable alterations in haemato-biochemical parameters were recorded in pyometra affected dogs as compared to healthy dogs. Moreover, the significant alterations ($P \leq 0.05$) were recorded in the mean values of total leucocyte count, platelet count, neutrophil percent, ALT, AST, ALP, BIT and BID, GGT, CRE and BUN. All the haemato-biochemical parameters were comparable to their respective reference values after either medicinal treatment or ovariohysterectomy of dogs.

Haematological examination revealed a decrease in the total erythrocyte count, haemoglobin, and packed cell

volume in all pyometra animals in comparison to control values. Though these findings are not consistent with those of earlier authors who purport that, anaemia in pyometra affected bitches results from erythrocyte diapedesis into the lumen of the uterus, and toxic depression of erythropoiesis in the bone marrow (Borresen and Skrede, 1980; Johnson, 1998; Root Kustritz, 2005; Tanja et al., 2006). In this study, leucocytosis with neutrophilia was a consistent finding. This type of leucogram has also been reported in other studies and is indicative of bone marrow stimulation by inflammatory processes, stress response, and/or acute phase reaction (Johnson et al., 2001 and Root Kustritz, 2005). The number of band cells per microscopic field was also higher in animals with pyometra (Figure 4) which is also reported by Reny et al.



Figure 5. Thick and sanguinus vaginal discharge in a case of open pyometra.



Figure 6. Ultrasonogram showing multiple anechoic pockets separated by thick fibrous bands and a thickened uterine wall from closed pyometra.

(2002). De Schepper et al. (1987) and Stone et al. (1988) have opined that the most characteristic inflammatory leucogram has a marked elevation of the total white blood cell count and usually a regenerative left shift in the differential white cell count. These changes were noted in

all pyometra cases.

Biochemically, there were significant elevations of serum ALT, AST, ALP, BIT, BIT, BID and GGT in all dogs with pyometra, which were reduced to their respective reference values after either medicinal treatment or

Table 1. Mean±SD of haematological parameters in bitches from different groups.

Parameter	Control	Pre treatment	Pre surgery	Post treatment	Post surgery
HG (g/dl)	13.42±0.76	9.32±2.51	7.38±2.16*	12.14±2.14	11.07±1.70
TEC (×10 ⁶ /μl)	6.67±0.71	4.85±1.47	3.77±1.12*	6.31±0.94	5.21±1.14
PCV (%)	40.15±1.88	29.73±8.48	24.18±6.85*	37.46±6.14	36.2±4.87
MCH (pg)	20.28±1.92	19.19±3.20	19.19±3.49	20.04±2.26	19.33±1.46
MCHC (g/dl)	33.39±0.58	30.68±2.27	30.57±1.89	31.7±0.58	31.16±1.65
PLT (×10 ⁵ /μl)	156.4±21.90	197.4±71.24*	216.3±102.06*	258.8±76.68*	221.62±75.44*
TLC (×10 ³ /μl)	8.02±1.67	30.45±21.72*	29.29±27.18*	15.09±8.40	8.65±1.96
N (%)	67±5	82.4±4.47*	86.2±3.73*	66.5±6.71	77.12±4.91
L (%)	29.5±5.33	13.3±3.37	8.9±2.84	29.7±7.08	15.4±9.87
M (%)	2.5±0.67	3.5±3.23	4.4±2.49	2.7±2.05	4.2±3.62
E (%)	1±1	0.8±1.07	0.5±0.67	1.6±1.49	1.2±1.4
B (%)	0±0	0±0	0±0	0±0	0±0
BAND CELLS (/field)	0.3±0.45	3.9±0.83*	3.4±2.2*	0.7±0.64	0.75±0.82

*Statistically significant differences compared to the control group (P≤ 0.05) (Snedecor and Cochran, 1994).

Table 2. Mean±SD of biochemical changes in bitches from different groups.

Parameter	Control	Pre treatment	Pre surgery	Post treatment	Post surgery
ALT (IU/L)	20.4±2.32	71.86±21.95*	99.16±44.01*	58.04±14.44*	39.8±17.91*
AST (IU/L)	21.67±2.92	50.81±17.22*	54.02±23.94*	51.68±15.82*	21.06±2.19
ALP (IU/L)	9.50±2.23	102.33±46.03*	212.4±69.25*	51.80±34.16*	32.8±12.28
TPP (g/dl)	6.8±0.99	4.78±0.89	4.28±0.87	7.32±1.23	7.62±0.42
ALB (g/dl)	2.63±0.62	2.57±0.57	1.65±0.48*	3.21±0.48	2.68±0.35
BIT (mg/dl)	0.10±0.02	1.42±0.61*	2.39±0.48*	0.94±0.16	0.96±0.26
BID (mg/dl)	0.56±0.08	0.32±0.26	1.15±0.40*	0.06±0.12	0.18±0.23
GGT (IU/L)	4.52±1.68	11.74±9.99*	20.41±4.36*	5.45±0.79*	7.95±2.02
CRE (mg/dl)	0.57±0.29	2.38±1.11*	2.50±0.71*	1.64±0.52	1.53±0.20
BUN (mg/dl)	10.94±2.25	36.45±22.86*	49.51±14.48*	23.25±7.32	19.92±2.82

*Statistically significant differences compared to the control group (P≤ 0.05) (Snedecor and Cochran, 1994).

ovariohysterectomy of dogs. Though, these enzymes thought to be markedly sensitive and nonspecific to conclude in multiple types of tissue degradation needs more specific tools to summarise specific diagnosis of pathologically involved systems. In cholestasis, from membranes of hepatocytes GGT and ALP are synthesised and released in hepatocellular damage and they are made soluble. GGT leaks out from the bile duct cells due to pressure from inside the bile ducts results into elevation. In the later stages, as a secondary effect of cholestasis AST, ALT and bilirubin may be elevated due to hepatic parenchymal damage (Ko et al., 2005; EASL Clinical Practice Guidelines, 2009). Possible explanations for observed increase in all these parameters include hepatocellular damage caused by septicaemia, changes in hepatic circulation and cellular hypoxia in dehydrated bitches, and intrahepatic cholestasis; which have been reported by many researchers like Borresen and Skrede (1980), Wheaton et al. (1989) and Ravishankar et al. (2004). Fransson et al. (1997) reported a high level of serum ALP during haemato-biochemical studies of pyo-

metra dogs similar to these findings. Sodikoff et al. (1995) have stated that major causes of increased serum ALP activity in dogs are cholestatic liver diseases.

A constant finding in all of the pyometra cases was a decrease in plasma protein and serum albumin concentration. Following medicinal treatments or surgical ovariohysterectomy, these values were increased and comparable to normal values due to immediate body reactions to treatment and surgery in recovery phase during initial follow ups. Borresen and Skrede (1980) have opined that plasma protein changes in pyometra dogs are the result of acute phase reactions. Fantoni et al. (1999) have stated that sepsis and endotoxemia cause increased vascular permeability and contribute to loss of protein. Earlier, Ravishankar et al. (2004) reported a hypoalbuminemia and hyperglobulinemia in cases of pyometra with total plasma protein concentration remaining unchanged. In the study, however, it was observed as a decrease in total plasma protein and serum albumin in all dogs with pyometra which may be attributed to their dehydrated status. Increased BUN levels in pyometra ca-

ses was observed, which then decreased following treatment as the condition of the animal improved. Similar to our findings, Jayathangaraj et al. (1994) and Ravishankar et al. (2004) recorded increased BUN levels in dogs with pyometra; however, Renton et al. (1971), Renton et al. (1993) and Troxel et al. (2002) recorded normal BUN levels pyometra cases. An increased BUN level can be associated with prerenal, renal or post renal causes. An increase in serum creatinine values was recorded in all cases of pyometra, which is in agreement with the findings of Stone et al. (1988) and Ravishankar et al. (2004). These authors have postulated that elevated BUN and CRE levels might be due to organic tissue destruction observed in pyometra. The concentration of GGT was also elevated in dogs with pyometra. GGT is an induced liver enzyme that indicates disease of the portal biliary system and increased GGT activity suggests cholestatic liver disease in dogs.

Thus, all dogs with pyometra had significant clinicopathological abnormalities (viz.; prominent symptoms like vaginal discharges, abdominal distentions, ultrasonographic evaluations, haemato-biochemical parameters, differential leucocyte count, band cell numbers) indicative of either hepatic or renal dysfunction. Nevertheless, this hepato-renal dysfunction appears to be of reversible nature as all the haemato-biochemical parameters studied were comparable to their respective reference values after either medical treatment or surgical ovariohysterectomy during follow ups in recovery period. This can suggest proper guidelines to include essential parameters for diagnosis of pyometra and to evaluate condition of patients in order to design treatment regime for better improvement in health of pyometric bitches.

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