

Hemato-biochemical and inflammatory features in canine pyometra

Anusha K.^{1*}, Sadasiva Rao K.², Srinivas M.², Sreenu M.³ and Aswani Kumar K.⁴

¹Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati- 517 502, Andhra Pradesh, India; ²Department of Veterinary Gynaecology and Obstetrics, NTR College of Veterinary Science, Gannavaram- 521 102, Andhra Pradesh, India; ³Department of Veterinary Surgery and Radiology, NTR College of Veterinary Science, Gannavaram- 521 102, Andhra Pradesh, India; ⁴Department of Veterinary Biochemistry, NTR College of Veterinary Science, Gannavaram- - 521 102, Andhra Pradesh, India

Abstract

The study aimed to assess the changes in hemato-biochemical profiles and serum C-reactive protein (CRP), serum amyloid A (SAA) concentrations in pyometra-affected bitches and healthy control. The hematological parameters viz., hemoglobin (Hb), packed cell volume (PCV) and total erythrocyte count (TEC) were significantly lower in pyometra-affected bitches compared to healthy controls. The total leucocyte count (TLC), percentage of neutrophils, band neutrophil count, total protein, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and creatinine were significantly ($P<0.01$) elevated, while, albumin and albumin/globulin ratio (A/G) were significantly ($P<0.01$) lowered in pyometra than healthy controls. The concentration of two major acute phase proteins viz., CRP (71.12 ± 6.19 vs 3.60 ± 1.33 mg/L) and SAA (60.68 ± 2.27 vs 1.76 ± 0.17 mg/L) were significantly ($P<0.01$) higher in pyometra when compared to healthy control bitches.

Keywords: Canine, C- reactive protein, Pyometra, Serum amyloid A

Highlights

- Anemia, leukocytosis with left shift, hyperproteinemia and azotemia were the significant findings in canine pyometra.
- The concentration of two major acute phase proteins viz., CRP and SAA were significantly elevated in pyometra.

Pyometra is a common reproductive problem in sexually intact bitches. Most often occurs four weeks to four months after the onset of estrus (Smith, 2006). Though the etiology was not fully understood, the disease primarily involves hormonal changes and infectious agents which complicate the process. The disease may result in a life-threatening condition, which requires an early diagnosis and immediate medical attention to conserve the life of the animal (Hagman, 2018). The present study was undertaken to assess the changes in hemato-biochemical profiles and serum CRP, SAA concentrations in pyometra-affected bitches and healthy control.

The study comprised of 75 bitches of various breeds aged between two to fifteen years suspected to be suffering from pyometra presented to the Department of Veterinary Gynaecology and Obstetrics, NTR College of Veterinary Science, Gannavaram. All the dogs were subjected to a detailed clinical examination. The preliminary diagnosis of pyometra was made based on case history, physiological parameters (temperature, appearance of mucous membranes, hydration status, demeanor, abdominal pain and palpable enlarged uterus) and diagnostic imaging by either abdominal ultrasonography

or radiography, or both. Clinically healthy bitches (n=10) in anestrus brought for elective spaying underwent routine gynaecological examination, and blood tests served as control in the study. Blood samples were collected aseptically using sterile EDTA vacutainers from cephalic and/or saphenous vein. Further blood (5 mL) was collected in a sterile vial with clot activator. The serum was separated by centrifugation at 3000 rpm for seven min. Hematological tests viz., DLC, TLC, Hb, PCV, TEC and biochemical tests viz., ALT, AST, total protein (TP), albumin, A/G, BUN and creatinine were performed by adopting standard methods. C-reactive protein (CRP) estimation was done by using a latex agglutination kit used for humans, as per the manufacturer's recommendations (C-Reactive Protein, BioSystems, Barcelona, Spain). Canine Serum amyloid A (GENLISATM, Krishgen Biosystems, Mumbai, India) sandwich ELISA commercial kit was used for the quantitative determination of canine serum amyloid A (SAA). The data generated in the study was tabulated and subjected to statistical analysis by using the SPSS (Statistical Product and Service Solutions) 20.0 version as per the methods described by Snedecor and Cochran (1994).

*Corresponding Author, E-Mail: vetdoc04@gmail.com

The most common symptoms reported by owners were the presence of vaginal discharge (80.00%), followed by polydipsia (74.60%), inappetence (72.00%), vomiting (68.00%), diarrhoea (12.00%) and polyuria (57.30%). Presence of vomiting and diarrhoea could be due to the adverse effects of higher levels of endotoxins as opined by Hardie and Kruse-Elliott (1990). Polydipsia and polyuria had been considered as classical signs of pyometra and indicated renal involvement in the disease and are considered as the common symptom in pyometra (Jitpean *et al.*, 2014; Bhat *et al.*, 2018; Samantha *et al.*, 2018).

The physiological and hematological parameters in pyometra affected and control bitches were presented in Table 1. The mean temperature ($P<0.01$) and respiratory rate ($P<0.05$) were significantly higher in pyometra affected bitches. It was evident from the present study that normal, subnormal or elevated temperature could be observed in bitches suffering from pyometra. In the present

study, a slightly higher percentage of bitches had pale (28.00%) mucous membranes due to anaemia. Several authors reported that bitches with pyometra had pale (10.90 to 20.0%) or hyperaemic (16.30 to 21.00%) mucous membranes (Jitpean *et al.*, 2014; Kitshoff *et al.*, 2015; Bostrom, 2018). The findings on percentage of bitches with dehydration and depression were similar to those reported previously by Hagman *et al.* (2011), Jitpean *et al.* (2014), Bhat *et al.* (2018) and Bostrom (2018).

The Hb ($P<0.05$), PCV ($P<0.01$) and TEC ($P<0.01$) were significantly lower, whereas TLC and percentage of neutrophils were significantly ($P<0.01$) higher in pyometra affected bitches compared to control. Diapedesis of erythrocytes into the lumen of uterus, reduced life span of circulating erythrocytes and toxic depression of erythropoiesis in the bone marrow might have resulted in anaemia (Kustritz, 2005). Leucocytosis with a relative increase in neutrophils, band neutrophils and decreased

Table 1. Physiological and hematological parameters of control and pyometra bitches

Sl. No.	Parameter	Control (n=10)	Pyometra (n=75)	P-value
1	Temperature (°F)	101.98±0.08 ^a	102.41±0.11 ^b	0.004**
2	Heart rate (beats/min)	102.90±1.97	109.16±1.46	0.19
3	Respiratory rate (breaths/min)	22.80±0.89 ^a	26.01±1.06 ^b	0.026*
4	Hemoglobin (g/dL)	11.45±0.30 ^b	10.05±0.24 ^a	0.023*
5	PCV (%)	39.80±0.88 ^b	31.45±0.81 ^a	0.007**
6	TEC (x10 ⁶ /μL)	6.83±0.22 ^b	5.41±0.13 ^a	0.009**
7	TLC (x10 ³ /μL)	10.27±0.42 ^a	20.89±1.13 ^b	0.00**
8	Neutrophils (%)	68.10±1.32 ^a	86.67±0.70 ^b	0.00**
	Band neutrophils (%)	0.0±0.0 ^a	19.17±1.07 ^b	0.00**
	Lymphocytes (%)	28.20±1.49 ^b	10.80±0.58 ^a	0.00**
	Monocytes (%)	2.00±0.26	1.61±0.16	0.223
	Eosinophils (%)	1.70±0.37	1.04±0.14	0.119

Values with different superscripts (a, b) within a row differ significantly (* $P<0.05$, ** $P<0.01$)

Table 2. Serum biochemical and inflammatory parameters of control and pyometra bitches

Sl. No.	Parameter	Control (n=10)	Pyometra (n=75)
1	Total protein (mg/dL)	6.29±0.17 ^a	7.52±0.17 ^b
2	Albumin (mg/dL)	3.39±0.1 ^b	2.70±0.07 ^a
3	Globulin (mg/dL)	2.89±0.17 ^a	4.82±0.18 ^b
4	A/G ratio	1.21±0.09 ^b	0.63±0.03 ^a
5	ALT (U/L)	25.02±2.19 ^a	37.00±2.11 ^b
6	AST (U/L)	24.06±1.75 ^a	47.60±2.41 ^b
7	ALP (U/L)	50.22±3.27 ^a	138.61±9.57 ^b
8	BUN (mg/dL)	13.26±0.95 ^a	48.30±5.31 ^b
9	Creatinine (mg/dL)	0.78±0.07 ^a	2.41±0.26 ^b
10	CRP (mg/L)	3.60±1.33 ^a	71.12±6.19 ^b
11	SAA (mg/L)	1.76±0.17 ^a	60.68±2.27 ^b

Values with different superscripts (a, b) within a row differ significantly ($P<0.01$)

lymphocytes observed in pyometra affected bitches from the present study had been considered a classical sign of pyometra by several authors (Dabrowski *et al.*, 2015; Jitpean *et al.*, 2017; Samantha *et al.*, 2018).

As presented in Table 2, The bitches with pyometra had significantly ($P < 0.01$) elevated TP, globulin, ALT, AST, ALP, BUN, creatinine, CRP and SAA compared to healthy control. Hyperproteinaemia with marked increase in globulin over albumin fraction in pyometra-affected bitches could be due to an acute phase reaction and synthesis of antibodies in response to bacterial infection as opined by Borresen and Skrede (1980) and Hagman (2004). The elevated levels of ALP might be due to hepato-cellular hypoxia caused by toxemia and intrahepatic cholestasis (Borresen and Skrede, 1980; Verstegen *et al.*, 2008). BUN and creatinine are catabolic waste products that are normally excreted by the kidneys, and therefore both are good indicators of glomerular filtration rate and are negatively correlated with it (Neel and Grindem, 2000). Elevated serum CRP and SAA

concentrations reflected the activation of the immune system and could be used to evaluate the health status of pyometra affected bitches, as confirmed in our study. Based on the results from the present study, the serum CRP and SAA concentrations could be used to assess the severity of inflammation in canine pyometra (Dabrowski *et al.*, 2007).

Conflict of interest: Authors have no conflict of interest in this study.

Author's contributions: AK: Collected and analyzed the samples, and drafted the manuscript; SRK, SM: Conceived and designed the study. SM: Helped in surgical procedures and supervised the research. AKK: Helped in biochemical and biomarker analysis.

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REFERENCES

- Bhat FH, Sharma U, Pande N, Pandey AK and Mudasir M, 2018. Incidence of canine pyometra in an around Jammu region. *J Pharm Innov*, 7(11): 192-196
- Borresen B and Skrede S, 1980. Pyometra in the dog- A pathophysiological investigation. V. The presence of intrahepatic cholestasis and an "acute phase reaction". *Nord Vet Med*, 32: 378-386
- Bostrom NJ, 2018. A retrospective study of prognostic markers in bitches diagnosed with pyometra. Master Thesis of Integrated Studies of Veterinary Medicine, Lithuanian University of Health Sciences, Kaunas
- Dabrowski R, Szczubial M, Kostro K, Wawron W, Ceron JJ *et al.*, 2015. Serum insulin-like growth factor-I and C-reactive protein concentrations before and after ovariohysterectomy in bitches with pyometra. *Theriogenology*, 83(4): 474-477, doi: 10.1016/j.theriogenology.2014.09.024
- Dabrowski R, Wawron W and Kostro K, 2007. Changes in CRP, SAA and haptoglobin produced in response to ovariohysterectomy in healthy bitches and those with pyometra. *Theriogenology*, 67(2): 321-327, doi: 10.1016/j.theriogenology.2006.07.019
- Hagman R, 2004. New aspects of canine pyometra- studies on epidemiology and pathogenesis. Ph.D. Thesis, Department of Small Animal Clinical Science, Swedish University of Agricultural Science, Uppsala, Sweden
- Hagman R, 2018. Pyometra in small animals. *Vet Clin North Am Small Anim Pract*, 48(4): 639-661, doi: 10.1016/j.cvsm.2018.03.001
- Hagman R, Lagerstedt AS, Hedhammar A and Egenvall A, 2011. A breed-matched case-control study of potential risk- factors for canine pyometra. *Theriogenology*, 75(7): 1251-1257, doi: 10.1016/j.theriogenology.2010.11.038
- Hardie EM and Kruse-Elliott K, 1990. Endotoxic shock: Part I: A review of causes. *J Vet Intern Med*, 4(5): 258-266, doi: 10.1111/j.1939-1676.1990.tb03119.x
- Jitpean S, Ambrosen A, Emanuelson U and Hagman R, 2017. Closed cervix is associated with more severe illness in dogs with pyometra. *BMC Vet Res*, 13(11): 1-7, doi: 10.1186/s12917-016-0924-0
- Jitpean S, Strom Holst B, Emanuelson U, Hoglund OV, Pettersson A *et al.*, 2014. Outcome of pyometra in female dogs and predictors of peritonitis and prolonged postoperative hospitalization in surgically treated cases. *BMC Vet Res*, 10(6): 1-12, doi: 10.1186/1746-6148-10-6
- Kitshoff A, Van Goethem B, Boyen F, Tas O, Polis I *et al.*, 2015. Clinical parameters as predictors of bacterial isolation in the uterine content of dogs suspected of pyometra. *Vlaams Diergeneesk Tijdschr*, 84(4): 188-196, doi: 10.21825/vdt.v84i4.16594
- Kustritz RMV, 2005. Cystic endometrial hyperplasia and pyometra. In: *Text book of Veterinary Internal Medicine*, 6th edn, (ed S G Ettinger and E C Feldman), Elsevier Saunders, St. Louis, pp 1676-1680
- Neel JA and Grindem CB, 2000. Understanding and evaluating renal function. *Vet Med*, 95(7): 555- 565
- Samantha G, Sarath T, Monica G, Arunmozhi N, Sridevi P *et al.*, 2018. Ultrasonographic and haemato-biochemical evaluation of bitches affected with cystic endometrial hyperplasia-pyometra complex. *Int J Curr Microbiol Appl Sci*, 7(6): 2327-2338, doi: 10.20546/ijcmas.2018.706.278
- Smith FO, 2006. Canine pyometra. *Theriogenology*, 66(3): 610-612, doi: 10.1016/j.theriogenology.2006.04.023
- Snedecor GW and Cochran WG, 1994. *Statistical Methods*. 7th edn, Oxford and JBH Publishing, New York, USA
- Verstegen J, Dhaliwal G and Verstegen OK, 2008. Mucometra, cystic endometrial hyperplasia, and pyometra in the bitch: advances in treatment and assessment of future reproductive success. *Theriogenology*, 70(3): 364-374, doi: 10.1016/j.theriogenology.2008.04.036

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