The Pharma Innovation Journal 2022; SP-11(5): 1936-1939

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(5): 1936-1939

www.thepharmajournal.com
Received: 08-03-2022
Accepted: 12-04-2022

Shravya BLG
M.V.Sc Scholar,
Department of Veterinary Gynaecology and Obstetrics
Veterinary College, KVAFSU,
Karnataka, India

Ravindranath BM
Associate Professor and Head,
Department of Veterinary Gynaecology and Obstetrics,
Veterinary College, KVAFSU,
Karnataka, India

Sahadev A
Professor and Head,
Department of Veterinary Gynaecology and Obstetrics
Veterinary College, KVAFSU,
Karnataka, India

Kalmath GP
Associate Professor,
Department of Veterinary Physiology, Veterinary College,
KVAFSU, Karnataka, India

Renukaradhya GJ
Assistant Professor,
Department of Veterinary Gynaecology and Obstetrics
Veterinary College, KVAFSU,
Karnataka, India

Suchitra BR
Assistant Professor,
Department of Veterinary Gynaecology and Obstetrics
Veterinary College, KVAFSU,
Karnataka, India

Corresponding Author
Ravindranath BM
Associate Professor and Head,
Department of Veterinary Gynaecology and Obstetrics,
Veterinary College, KVAFSU,
Karnataka, India

Haemato-biochemical, ultrasonographic and histopathological changes associated with canine pyometra

Shravya BLG, Ravindranath BM, Sahadev A, Kalmath GP, Renukaradhya GJ and Suchitra BR

Abstract
The study was conducted to ascertain the haemato-biochemical, ultrasonographic and histopathological changes associated with cystic endometrial hyperplasia (CEH)-pyometra complex in dogs and also to correlate uterine wall thickness with haemato-biochemical and histopathological changes. Dogs presented to clinic at the Department of Veterinary Gynaecology and Obstetrics were selected for the present study as control (Group I) and pyometra group (Group II) which were subjected to ultrasonographic examination and haemato-biochemical evaluation. Uterine tissue samples collected after ovariohysterectomy were subjected for histopathological evaluation. On trans-abdominal ultrasonography, an increase in the uterine wall thickness (4.28±0.32 mm) and uterine horn diameter (32.85±13.75 mm) was observed in CEH- pyometra cases. Absolute leukocytosis with neutrophilia and lymphopenia were prominent hematological changes whereas, elevation in BUN, creatinine, ALT and total protein level along with hypoalbuminaemia were significant findings in serum biochemical profile of dogs with CEH- pyometra complex. Considerable alterations were found in the histological architecture of uterine tissue in CEH- pyometra affected dogs compared to control group animals. However, there was no significant correlation between uterine wall thickness and haemato-biochemical profile as well as histopathological changes in CEH- pyometra affected dogs in the present study.

Keywords: CEH- Pyometra complex, trans-abdominal ultrasonography, ovariohysterectomy, histopathology

Introduction
Pyometra is one of the most critical and frequent diseases of intact female dogs affecting more than half of their population and if left untreated, mortality rate can reach up to 3-10 per cent [1]. Pyometra is characterized by uterine bacterial infection and inflammation with pus accumulating in the uterus. Till date, pathophysiology of pyometra has not been completely understood. While cystic endometrial hyperplasia (CEH) is present in the majority of dogs with pyometra, some dogs may develop pyometra without showing any evidence of CEH. The pathophysiological alterations in the uterus and the haemato-biochemical changes that occur during pyometra have been recommended as prognostic indications for pyometra-affected dogs [2]. While ultrasonography remains as an efficient way to diagnose pyometra, histopathology gives a greater insight to the damage that has happened to the uterine wall by the infection and inflammation [3]. The present study was conducted to ascertain the haemato-biochemical, ultrasonographic and histopathological changes associated with CEH- pyometra in dogs, further to correlate uterine wall thickness with haemato-biochemical and histopathological changes.

Materials and Methods
Dogs presented to clinic at the Department of Veterinary Gynaecology and Obstetrics were selected for the present study. Ten healthy diestrual dogs served as control animals (Group I) whereas, twenty CEH- pyometra affected dogs (Group II) were further subdivided into Group II-A and Group II-B based on the mean uterine wall thickness. Dogs from all the groups were subjected to B-mode, real time ultrasound scanning (PROSOUND ALPHA, Japan) equipped with curvilinear multi-frequency (5.0 MHz) probe to assess the uterine wall thickness and the distension of uterine horns. The mean uterine wall thickness of animals in Group II was calculated and further subdivision of the group was done as Group II-A, which included animals with uterine wall thickness below or equal to the average (n=9) and Group II-B, which

included animals with uterine wall thickness above the average (n=11).

Blood samples were collected from all the animals before ovariohysterectomy. Haemato-biochemical parameters included total erythrocyte count (TEC), total leucocyte count (TLC), differential leukocyte count (DLC), haemoglobin (Hb), packed cell volume (PCV), platelet count, blood urea nitrogen (BUN), creatinine, albumin, globulin, total protein (TP) and alanine amino transferase (ALT) were determined using semi auto analyzer. Uterine tissue samples collected from all the animals after ovariohysterectomy were subjected for histopathological evaluation.

The data obtained in the study was tabulated and subjected to t- test to assess significant differences among means, where $P \leq 0.05$ was considered as statistically significant. This statistical analysis was done with the help of SPSS (version 16.0) statistical software.

Results and Discussion

The results of the present study has been represented in Table 1 and 2. There was significant increase ($P<0.05$) in the TLC and neutrophil percentage of group II dogs that was in line with Shah *et al*. (2020), Singh *et al*. (2020) and Vijay *et al*. (2021) [4, 5, 6] attributable to higher amount of inflammatory response initiated by the diffused suppurative inflammation of uterus [7]. Mild anemia observed in the animals of Group II was in agreement with the findings of Babu *et al*. (2018), Maharathi *et al*. (2020), Rosa filho *et al*. (2020) and dos Anjos *et al*. (2021) [8, 9, 10, 11]. Normocytic, normochromic anaemia observed in pyometra affected dogs is due to the toxic effects of uterine contents on the bone marrow, lack of available iron and diapedesis of erythrocytes to the uterus [12]. Animals with renal dysfunction due to endotoxaemia most likely produce less erythropoietin, further worsening the anemia [11]. Significant decrease ($P<0.05$) in the lymphocyte percentage of Group II dogs was in accordance with Plavec *et al*. (2006) and Lakshmikanth (2016) [13, 14], which might be attributed to the severe stress during the disease. Further, lymphopenia occurs due to immunosuppression and inhibition of mitogen driven lymphocyte proliferation during pyometra [15].

Significant increase ($P<0.05$) in the BUN, serum creatinine, TP and ALT of Group II dogs was observed in the present study which was in agreement with the previous reports by Shah *et al*. (2017) [17]. Significant decrease ($P<0.05$) in the serum albumin was found in the Group II dogs compared to Group I dogs similar to the findings of Renukaradhya (2011), Shah *et al*. (2017), Lee *et al*. (2016) [16, 17, 18], owing to renal dysfunction due to deposition of the immune complexes along the glomerular basement membrane which allows leakage of plasma albumin. The present findings of marked increase in globulin over albumin fraction in pyometra dogs could be due to an acute phase reaction and synthesis of antibodies in response to bacterial infection [12].

There was significant increase ($P<0.05$) in the uterine wall thickness of animals of Group II (4.28±0.32mm) when compared to control dogs (2.03±0.22mm) which was in line with Rautela and Katiyar (2009), Ahuja *et al*. (2019) and Singh *et al*. (2020) [19, 20]. There was no significant difference between haemato-biochemical parameters of Group II-A and Group II-B dogs suggesting no influence of mean uterine wall over the haemato- biochemical values of pyometra affected dogs (Table 2). The present findings were in conformity with the observations of De Bosschere *et al*. (2001) [21] who opined that CEH and pyometra were separate ailments and could prevail independently. The current findings were in contrast to the observations of Manokaran *et al*. (2014) and Manokaran *et al*. (2018) [22, 23], who found a proportionate increase in the biochemical parameters as the uterine wall thickness increased.

The uterine tissue from control animals showed intact smooth endometrial lining with columnar cells and presence of endometrial glands in the endometrial stroma without any pathological changes. Whereas, Group II- A animals had cystic endometrial hyperplasia and endometrial proliferation along with edema of stroma. Infiltration of neutrophils, lymphocytes, plasma cells and macrophages in the stroma and glandular lumen were found in all cases. Vacuolation and desquamation of glandular epithelium, eosinophilic or basophilic secretions in the glandular lumen, fibrous proliferation of endometrial stroma and adenomyosis were observed in few cases. Group II-B animals also had similar lesions except for marked villous proliferation of the endometrium and squamous metaplasia in few cases. The histopathological observations made in the present study were in line with Dow (1959), Bigliardi *et al*. (2004), Jena *et al*. (2015), Rosa filho *et al*. (2020) and Wozna-Wysocka *et al*. (2021) [3, 10, 24, 25, 26]. However, no correlation between the thickness and the histopathological changes was noticed in the present study which is in agreement with Sur and Chakravorty (2016) [27].

Table 1: Haemato-biochemical profile of control and pyometra affected dogs (Mean ± SE)

Parameters	Control (n=10)	CEH- pyometra (n=20)
TEC (x10^6/cmm)	6.46 ± 0.55	5.46 ± 0.25
Hb (g/dL)	11.65 ± 0.56	11.38 ± 0.59
PCV (%)	35.47 ± 1.62	36.12 ± 1.77
TLC (x10^3/cmm)	7.48 ± 0.53^a	47.52 ± 7.00^b
Neutrophil (%)	70.57 ± 1.29^a	83.46 ± 1.06^b
Lymphocyte (%)	27.89 ± 1.19^b	12.5 ± 0.93^a
Monocyte (%)	1.54 ± 0.38	2.67 ± 0.62
Eosinophil (%)	0.00 ± 0.00	1.37 ± 0.32
Platelet (x10^3/cmm)	239.67 ± 28.02	202.4 ± 21.96
TP (g/dL)	6.24 ± 0.29^a	7.38 ± 0.20^b
Albumin (g/dL)	3.02 ± 0.20^b	2.28 ± 0.07^a
Globulin (g/dL)	3.22 ± 0.18^a	5.15 ± 0.22^b
BUN (mg/dL)	15.22 ± 3.05^a	30.50 ± 5.35^b
Creatinine (mg/dL)	0.97 ± 0.11^a	2.73 ± 0.51^b
ALT (U/L)	25.24 ± 4.10^a	36.30 ± 2.49^b

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

Table 2: Hemoto-biochemical profile in Group II-A and Group II-B dogs (Mean ± SE)

Parameters	CEH- pyometra affected dogs	
	Group II-A (n=9)	Group II-B (n=11)
TEC (x10^6/cmm)	5.02 ± 0.35	5.82 ± 0.34
Hb (g/dL)	10.50 ± 0.70	12.11 ± 0.89
PCV (%)	33.78 ± 1.95	38.05 ± 2.73
TLC (x10^3/cmm)	54.82 ± 12.47	41.55 ± 7.71
Neutrophil (%)	83.81 ± 1.24	83.18 ± 1.70
Lymphocyte (%)	12.66 ± 0.85	12.37 ± 1.59
Monocyte (%)	2.17 ± 0.58	3.07 ± 1.05
Eosinophil (%)	1.37 ± 0.44	1.37 ± 0.48
Platelet (x10^3/cmm)	221.67 ± 20.58	186.64 ± 36.53
TP (g/dL)	7.26 ± 0.22	7.48 ± 0.33
Albumin (g/dL)	2.38 ± 0.07	2.20 ± 0.12
Globulin (g/dL)	4.99 ± 0.26	5.28 ± 0.36
BUN (mg/dL)	26.13 ± 8.07	34.07 ± 7.31
Creatinine (mg/dL)	2.32 ± 0.75	3.07 ± 0.71
ALT (U/L)	35.12 ± 1.88	37.28 ± 4.35

None of the values were significantly different ($P<0.05$)

This could possibly be due to diverse microscopic changes found in the CEH- pyometra affected uterus irrespective of the thickness of the uterine wall. In contrast, Manokaran *et al*. (2014) and Manokaran *et al*. (2018) [15, 16] reported that histopathological changes were proportionally higher or severe in thicker uterine wall than thinner ones.

Conclusion

Leukocytosis with neutrophilia and lymphopenia, elevation in BUN and total protein along with hypoalbuminaemia were the significant findings in dogs with CEH- pyometra complex in the present study. Thickness of the uterine wall showed variation in individual animals affected by CEH- pyometra and there was no relationship between the uterine wall thickness and haemato-biochemical profile of the CEH-pyometra affected dogs. Similarly, no correlation exists between the histopathological changes and uterine wall thickness in CEH- pyometra affected animals. However, there is a need for further studies in larger population of the animals to derive more accurate results.

Acknowledgement

Authors acknowledge Veterinary College, Bengaluru for the facilities and support to conduct the study.

Conflict of interest

Authors have no conflict of interest

References

1. Egenvall A, Hagman R, Bonnett BN, Hedhammar A, Olson P, Lagerstedt AS. Breed risk of pyometra in insured dogs in Sweden. Journal of veterinary internal medicine. 2001;15(6):530-8.
2. Thangamani A, Srinivas M, Chandra Prasad B. Pyometra in bitches: a critical analysis. International Journal of Science, Environment and Technology. 2018;7(3):1072-8.
3. Bigliardi E, Parmigiani E, Cavirani S, Luppi A, Bonati L, Corradi A. Ultrasonography and cystic endometrial hyperplasia-pyometra complex in the bitch. Reproduction in domestic animals. 2004;39(3):136.
4. Shah SA, Sood NK, Wani BM, Rather MA, Beigh AB, Amin U. Haemato-biochemical studies in canine pyometra. Journal of Pharmacognosy and Phytochemistry. 2017;6(4):14-7.
5. Singh LK, Patra MK, Mishra GK, Saxena AC, De UK, Singh SK, *et al*. Prospects of diagnostic and prognostic biomarkers of pyometra in canine. Asian Pacific Journal of Reproduction. 2020;9(4):166.
6. Vijay A, Shafiuzama M, Hemalatha S, Gokulakrishnan M. Haemato-biochemical changes in pyometra affected bitches during and after ovariohysterectomy. The Pharma Innovation Journal. 2021;10(4): 04-08.
7. Nath K, Tiwari SK, Sharda R, Garg S, Kalim MO. Pyometra induced biochemical changes in bitches. Indian veterinary journal. 2009;86(8):853-5.
8. Babu M, Sahadev A, Nethra R, Rashmi S. Clinical Studies on Haemato-Biochemical Attributes in Pyometra Affected Dogs. International Journal of Scientific Research. 2018;7(7): 902-904.
9. Maharathi SP, Dalai N, Mohapatra S, Mishra SR, Mahapatra AP, Kundu AK, *et al*. Haemato-Biochemical Alterations in Pyometra Affected Bitch. International Journal of Current Microbiology and Applied Science. 2020;9(6):1242-5.
10. Da Rosa Filho RR, Brito MM, Faustino TG, de Almeida LL, Manoel VC, Cogliati B, *et al*. Prostaglandin and antigestagen in pyometra bitches: vascular and stereological effect. Reproduction and Fertility. 2021;2(2):95-105.
11. dos Anjos MS, Bittencourt RF, Biscarde CE, de Andrade Silva MA, dos Santos ES, Junior LD, *et al*. Canine pyometra: interferences of age and type in blood count and serum biochemistry. Revista Brasileira de Ciencia Veterinária. 2021;28(3):167-173.
12. Hagman R. Clinical and molecular characteristics of pyometra in female dogs. Reproduction in domestic animals. 2012;47:323-5.
13. Plavec T, Celinšek B, Dolinar K, Pečar J, Nemec A, Butinar J. Haemostasis impairment in bitches with pyometra. Acta veterinaria. 2006;56(5-6):529-40.
14. Lakshmikanth TR. Hematological and biochemical studies in relation to severity in open and closed pyometra in bitches. Ph.D. thesis, 2016; KVAFSU, Bidar.
15. Faldyna M, Laznicka A, Toman M. Immunosuppression in bitches with pyometra. Journal of small animal practice. 2001;42(1):5-10.
16. Renukaradhya GJ. Studies on treatment of pyometra in bitches with antiprogestins. Ph.D. thesis, 2011. KVAFSU, Bidar.

17. Shah SA, Sood NK, Wani BM, Rather MA, Beigh AB, Amin U. Haemato-biochemical studies in canine pyometra. Journal of Pharmacognosy and Phytochemistry. 2017;6(4):14-7.
18. Lee JA, Kim IH, Hwang DY, Kang HG. Ultrasonography, Affected Age, Hematology and Clinical Signs according to Open or Closed Cervix in Dogs with Pyometra. Journal of Veterinary Clinics. 2016;33(6):362-7.
19. Rautela R, Katiyar R. Review on canine pyometra, oxidative stress and current trends in diagnostics. Asian Pacific Journal of Reproduction. 2019;8(2):45.
20. Ahuja AK, Honparkhe M, Sethi GS, Singh N, Jan F, Chauhan P. Association of canine pyometra with systemic inflammatory response syndrome. Journal of Entomology and Zoology Studies. 2019;7:1409-12.
21. De Bosschere H, Ducatelle R, Vermeirsch H, Van Den Broeck W, Coryn M. Cystic endometrial hyperplasia-pyometra complex in the bitch: should the two entities be disconnected. Theriogenology. 2001;55(7):1509-19.
22. Manokaran S, Ezakial Napolean R, Selvaraju M, Doraisamy KA, Balasubramaniam GA. Ultrasonographic, histopathological and hematological changes in bitches affected with pyometra. *In*: Proceedings of International Symposium on "Current challenges and translational research to augment animal reproduction", TANUVAS, Chennai, India. 2014, 218.
23. Manokaran S, Napolean RE, Selvaraju M, Balasubramaniam GA. A Comparative Study on Ultrasonographic and Bio-Chemical Changes with Histopthological Changes of Uterus in Pyometra Affected Bitches. International Journal of Livestock Research. 2018;8(12):188-193.
24. Dow C. The cystic hyperplasia-pyometra complex in the bitch. Journal of Comparative Pathology and Therapeutics. 1959;69:237.
25. Jena B, Rao KS, Das D, Reddy KC. Uterine histomorphological changes in canine pyometra. Journal of Cell and Tissue Research. 2015;15(1):4747.
26. Wozna-Wysocka M, Rybska M, Błaszak B, Jaskowski BM, Kulus M, Jaskowski JM. Morphological changes in bitches endometrium affected by cystic endometrial hyperplasia-pyometra complex–the value of histopathological examination. BMC Veterinary Research. 2021;17(1):1-11.
27. Chakravorty R. Correlation of endometrial thickness and histopathology in women with abnormal uterine bleeding. Reproductive System and Sexual Disorder. 2016;5(4):1-3.