The Pharma Innovation Journal 2023; SP-12(8): 360-362

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(8): 360-362

www.thepharmajournal.com
Received: 09-06-2023
Accepted: 19-07-2023

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Hematо-biochemical studies in open cervix pyometra affected dogs of breeding age

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Abstract
Pyometra is considered a diestrual disorder of aged intact bitches, but the condition has been reported in dogs of breeding age. Restoring the breeding value of bitches in such cases should be given importance rather than opting for usual ovariohysterectomy. The study was carried out in bitches brought to Small Animal Gynaecology and Obstetrics Out Patient Ward of Madras Veterinary College Teaching Hospital which were aged between 2 and 6 years. Twelve bitches diagnosed with open cervix pyometra were selected and grouped randomly into two treatment groups. In Group I (n= 6), bitches were treated with a combination cloprostenol @ 5µg/kg bwt subcutaneouly and Cabergoline @ 5µg/kg bwt orally once daily for 7 days and group II (n= 6) bitches treated with Dinoprost tromethamine intravaginally at the dose rate of 150 µg/ kg bwt for 7 days. Six apparently healthy bitches in dioestrus served as control group III. Blood and serum samples were collected from all the animals on day 0, 7, 14 and 21. Results revealed reduced PCV and platelet values, elevated WBC and Differential count in hematology. Serum biochemistry results recorded normal renal and hepatic profiles except for the serum bilirubin levels. Recovery rate of 83.3 and 66.6 percent was recorded in group I and II respectively. It is concluded that the bitches of young breeding age had comparatively normal renal profile in open cervix pyometra indicate that young breeding age may be a contributing factor to renal involvement.

Keywords: canine, open cervix pyometra, haemato-biochemical levels

1. Introduction
Pyometra is a common diestrual disorder of old intact bitches due to bacterial infection and consequent inflammation leading to accumulation of purulent exudate in the uterus (Nelson and Feldman, 1986) [2]. Susceptibility to pyometra depends greatly on age of animal, parity and breed. It usually affects adult intact bitches even though the same has been reported in animals of breeding age. Increased risk of pyometra was reported in nulliparous animals with a mean age of 8.5 years and the range from 1 to 18 years (Niskanen and Thrusfield, 1998) [1]. Pyometra developed in nearly 25% of bitches by 10 years age of which incidence was as high as 50% in some breeds and 10% in other breeds (Egenvall *et al.*, 2001) [3]. The studies of pyometra in the breeding age group is not recorded. In India, importing of pedigree dogs and dog breeding is in an accelerating phase. Even though surgery is considered as primary choice of treatment in pyometra, in breeding dogs medical management should be considered with equal importance. The present study emphasis on the treatment outcomes after medical management of pyometra and hematobiochemical alterations in pyometra affected bitches.

2. Materials and Methods
The study was carried out in bitches Small Animal Gynaecology and Obstetrics Out Patient Ward of Madras Veterinary College Teaching Hospital brought to were aged between 2 and 6 years. Twelve bitches diagnosed with open cervix pyometra were selected and grouped randomly into two treatment groups. In Group I (n= 6), bitches were treated with a combination cloprostenol @ 5µg/ kg bwt subcutaneouly and Cabergoline @ 5µg/kg bwt orally once daily for 7 days and group II (n= 6) bitches treated with Dinoprost tromethamine intravaginally at the dose rate of 150 µg/ kg bwt for 7 days. Six apparently healthy bitches in dioestrus served as control group III. Blood and serum samples were collected from all the animals on day 0, 7, 14 and 21.
The haematological parameters were assessed using blood from EDTA vial which include Haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC) and platelet count. Blood smear examination was done to detect the presence of blood parasites and for differential count.

Estimation of haematological parameters were done using automatic haematology analyser (Mindray, BC-2800). One sample of 2 ml blood in a clot activator serum vial was collected and centrifuged at 1500 rpm for 20 minutes for separation of serum samples. Serum biochemistry analysis was done using automatic analyser. Statistical analysis of data was performed by using computer software SPSS version 20 (International Business Machine (IBM) corp., Chicago, USA). Haematological and serum biochemical values were evaluated by Analysis of Variance and presented as Mean + SE.

3. Results and Discussion

Reduced RBC, platelet, PCV values were recorded while Hb levels were within the normal range on day 0. On day 21 no significant improvement was noticed in RBC and PCV values, however, platelet counts were improved. Hardy and Osborne (1974) [4] suggested that possible reasons for anaemia in dogs with pyometra could be due to toxic depression of bone marrow or loss of RBCs in to the uterine lumen. The possible reason could be due to improved appetite of the animals after treatment as well as reduction in the circulating levels of toxin in blood stream which would have reduced bone marrow suppression and intern toxic effects on bone marrow.

Thrombocytopaenia recorded with endotoxemia are mainly due to increased consumption of platelets in Disseminated Intravascular Coagulation (DIC) or endometrial bleeding, decreased production of platelets in the bone marrow due to bone marrow suppression (Dodds, 1989) [5] or Platelet Activating Factor (PAF) production mediated by bacterial production of endotoxin. PAF is released from leucocytes, platelets and endothelial cells. It could have resulted in activation and aggregation of thrombocytes as well as neutrophils (Kruth, 1998; Tsuchiya *et al*., 1999) [6, 7].

Leucocyte (x10^3 Cmm) was elevated on day 0 of treatment (16.35 + 1.80 and 25.38 + 4.86) which were returned to normal levels on day 21. Jitpean (2015) [9] and Shah *et al*. (2017) [10] reported that leucocytosis is a common feature in bitches with pyometra due to systemic infection. Jena *et al*. (2013) [8] opined that neutrophilia in pyometra might be due to presence of purulent exudates in uterus leading to chemotactic attraction of neutrophils and accelerated granulopoesis. Treatment to pyometra reduces the bacterial load in the uterine lumen as well as toxin levels in the blood stream so that neutrophil counts came back to normal levels after treatment. Mean lymphocyte count was within normal range on day 0 followed by lymphocytosis on day 21. Eosinophil and monocyte counts were with in normal range and unaltered.

Hepatic profile showed ALP, ALT, total protein and albumin levels were within range. Normal levels of creatinine and blood urea nitrogen were recorded in the present study. The observations were contradictory with the reports of Shah *et al*. (2017) [10]. He reported significant increase in BUN (63.6 ± 7.07) and creatinine (2.96 ± 0.6) values were found in pyometra affected bitches indicating that it may adversely affect the renal function and these changes were more evident in closed pyometra cases than open cervix pyometra cases. Renal function of the animals usually gets impaired in old age. Pyometra is more common in old animals. So along with endotoxins of gram-negative bacteria, age of the animal may be a contributing factor for the development of renal impairment. In present study, we included the animals between 2 and 6 years of age could be the reasons for an unaltered Blood Urea Nitrogen (BUN).

In the present study, almost the biochemical parameters are within normal reference range. Among the treated animals, one animal in group I and two bitches group II did not recover and were underwent ovariohysterectomy with an recovery rate of 83.3 and 66.6 percent recovery rate group I and II respectively. Three animals in group I and one animals in group II had return to estrus and mated.

Table 1: Haematological values in healthy control and open cervix pyometra affected bitches following two different medical treatment at weekly interval

		Day 0	Day 7	Day 14	Day 21	f-value	p-value
Haemoglobin (g/dL)	Group I	11.58 + 1.19	10.51 + 0.79	13.10 + 1.03	12.46 + 1.28	1.113^{NS}	0.368
	Group II	12.13 + 0.61	11.63 + 0.43	11.89 + 0.74	12.36 + 0.46	0.245^{NS}	0.864
	Group III	12.06 + 0.38	12.30 + 0.39	12.31 + 0.28	12.32 + 0.56	0.102^{NS}	0.958
	f-value	0.497^{NS}	1.841^{NS}	0.535^{NS}	0.064^{NS}		
	p-value	0.689	0.172	0.664	0.978		
Packed Cell Volume (percent)	Group I	$32.00 + 2.96^{b}$	$28.76 + 1.99^{b}$	$35.98 + 2.70^{ab}$	34.36 + 3.31	1.341^{NS}	0.291
	Group II	$33.68 + 1.58^{ab}$	$32.93 + 1.54^{b}$	$34.41 + 1.93^{ab}$	35.82 + 1.81	0.477^{NS}	0.702
	Group III	$38.53 + 0.51^{a}$	$38.33 + 0.70^{a}$	$38.31 + 0.42^{a}$	39.77 + 0.60	1.181^{NS}	0.345
	f-value	3.557^{*}	7.669^{**}	1.852^{*}	1.731^{NS}		
	p-value	0.033	0.001	0.045	0.201		
Red Blood Cells (m/mm^3)	Group I	$04.98 + 0.36$	$04.53 + 0.29$	$05.65 + 0.31^{a}$	05.02 + 0.47	1.758^{NS}	0.189
	Group II	04.82 + 0.32	04.63 + 0.22	$04.90 + 0.33^{ab}$	05.14+ 0.16	0.558^{NS}	0.649
	Group III	04.96 + 0.08	05.06 + 0.12	$05.03 + 0.11^{ab}$	05.12 + 0.10	0.366^{NS}	0.779
	f-value	0.255^{NS}	1.207^{NS}	2.419^{*}	0.053^{NS}		
	p-value	0.857	0.333	0.045	0.983		
White Blood Cells (x10^3) (Cmm)	Group I	$16.35 + 1.80^{abx}$	$16.016 + 3.66^{abx}$	$10.05 + 1.32^{bcxy}$	$8.74 + 0.53^{by}$	3.001^{*}	0.047
	Group II	$25.38 + 4.86^{bcx}$	$18.23 + 2.10^{axy}$	$16.36 + 1.94^{axy}$	$13.66 + 2.18^{ay}$	2.580^{*}	0.048
	Group III	$9.08 + 9.80^{c}$	$8.566 + 1.28^{b}$	$8.05 + 1.48^{c}$	$9.42 + 1.50^{ab}$	0.196^{NS}	0.898
	f-value	6.137^{**}	3.313^{*}	5.307^{**}	2.313^{NS}		
	p-value	0.004	0.041	0.007	0.115		
Platelets (x 10^4) (Cmm)	Group I	$13.57 + 3.89^{b}$	18.43 + 3.85	26.46 + 5.13	$21.88 + 5.84^{b}$	1.417^{NS}	0.269
	Group II	$21.85 + 4.62^{ab}$	25.61 + 5.17	24.39 + 4.09	$27.74 + 4.21^{ab}$	0.276^{NS}	0.842
	Group III	$34.01 + 4.05^{a}$	31.96 + 4.13	34.06 + 3.67	$40.22 + 1.58^{a}$	0.716^{NS}	0.555
	f-value	3.747^{*}	1.675^{NS}	1.041^{NS}	3.321^{*}		
	p-value	0.028	0.204	0.396	0.047		

Means bearing different superscript in each column and row differ significantly (p<0.05)

Table 2: Serum biochemical profile in healthy control and open cervix pyometra affected bitches following two different medical treatment at weekly interval

		Day 0	Day 7	Day 14	Day 21	f-value	p-value
Alkaline Phosphatase (ALP) (U/L)	Group I	086.00 + 18.79	116.16 + 16.41	122.50 + 13.46	125.40 + 15.24	1.259^{NS}	0.317
	Group II	124.00 + 15.94	155.66 + 07.13	130.66 + 08.90	152.50 + 18.26	1.379^{NS}	0.278
	Group III	101.66 + 07.54	099.66 + 13.58	109.66 + 13.05	117.50 + 13.44	0.397^{NS}	0.756
	f-value	1.985^{NS}	1.534^{NS}	0.282^{NS}	1.032^{NS}		
	p-value	0.149	0.236	0.898	0.403		
Alanine Aminotransferase (ALT) (U/L)	Group I	$43.33 + 11.67^{b}$	$36.83 + 08.37^{c}$	$52.00 + 06.36^{b}$	$60.60 + 06.56^{b}$	1.352^{NS}	0.287
	Group II	$55.16 + 06.54^{ab}$	$52.16 + 05.66^{bc}$	$61.00 + 06.68^{ab}$	$66.83 + 12.60^{ab}$	0.606^{NS}	0.619
	Group III	$77.00 + 09.90^{a}$	$88.33 + 08.99^{a}$	$78.16 + 09.10^{ab}$	$93.25 + 03.59^{a}$	0.707^{NS}	0.560
	f-value	2.029^{*}	3.931^{*}	2.793^{*}	2.347*		
	p-value	0.045	0.023	0.045	0.049		
Total Protein (g/dL)	Group I	07.40 + 0.16	$07.06 + 0.26^{ab}$	07.03 + 0.30	$06.92 + 0.16^{ab}$	0.750^{NS}	0.536
	Group II	07.38 + 0.43	$07.31 + 0.31^{a}$	07.05 + 0.23	$07.20+ 0.30^{a}$	0.194^{NS}	0.899
	Group III	06.41 + 0.25	$06.40 + 0.27^{b}$	06.53 + 0.17	$06.55 + 0.30^{ab}$	0.094^{NS}	0.962
	f-value	1.794^{NS}	2.893^{*}	0.815^{NS}	2.400^{*}		
	p-value	0.181	0.045	0.501	0.044		
Albumin (g/dL)	Group I	02.56 + 0.25	02.70 + 0.20	$02.90 + 0.11^{a}$	02.84 + 0.14	0.623^{NS}	0.609
	Group II	02.76 + 0.15	02.70 + 0.09	$02.93 + 0.11^{a}$	02.76+ 0.10	0.699^{NS}	0.563
	Group III	02.76 + 0.22	02.53 + 0.11	$02.63 + 0.12^{ab}$	02.65 + 0.10	0.403^{NS}	0.753
	f-value	0.573^{NS}	0.455^{NS}	4.112^{*}	0.307^{NS}		
	p-value	0.639	0.717	0.020	0.820		
Blood Urea Nitrogen (mg/dL)	Group I	14.54 + 1.54	15.99 + 3.79	14.62 + 2.17	13.11 + 2.00	0.198^{NS}	0.897
	Group II	13.40 + 1.50	13.83 + 1.66	14.85 + 1.04	14.42 + 1.00	0.231^{NS}	0.873
	Group III	13.80 + 1.01	15.15 + 1.48	14.61 + 1.35	16.57 + 0.86	0.718^{NS}	0.554
	f-value	3.044^{*}	0.181^{NS}	0.023^{NS}	1.110^{NS}		
	p-value	0.045	0.908	0.995	0.372		
Creatinine (mg/ dL)	Group I	1.25 + 0.25	0.99 + 0.191	1.070 + 0.24	1.02 + 0.18	0.262^{NS}	0.852
	Group II	1.04 + 0.09	0.89 + 0.076	1.103 + 0.08	0.81 + 0.10	2.077^{NS}	0.135
	Group III	0.88 + 0.08	0.90 + 0.133	0.718 + 0.07	1.07 + 0.15	1.485^{NS}	0.252
	f-value	0.792^{NS}	0.347^{NS}	1.292^{NS}	1.063^{NS}		
	p-value	0.513	0.792	0.304	0.391		

Means bearing different superscript in each column and row differ significantly ($p<0.05$)

4. Conclusion

Based on haemato-biochemical studies, it can be inferred that renal profile is not much unaltered in bitches with open cervix pyometra in young breeding age between 2 and 6 years of age, though haematological parameter got altered with varying degree. However, further investigations are being warranted in large number of bitches on recurrence of the condition, future fertility and fecundity after medical treatment of open cervix pyometra.

5. Acknowledgement

The authors are grateful to Tamil Nadu Veterinary and Animal Sciences University and all the faculty members for the support and help rendered to carry out this study.

6. Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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