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Research Article

Clinical, Bacteriological, and Histopathological Aspects of Endotoxic Pyometra in Bitches [1]

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Abstract

The object of this study was to compare the histopathologic change in uterus as well as the findings of clinical and bacteriological of twenty-four bitches with pyometra. Uterine lesions were grouped as acute endometritis, subacute endometritis, acute metritis, subacute metritis. Complete blood-count, blood biochemical parameters and toxin levels were determined. Only alkaline phosphatase levels were significantly different between all groups (P<0.01). Blood endotoxin level was different between the acute and subacute endometritis groups (P<0.01), and subacute endometritis and acute metritis groups (P<0.01). All bitches in the subacute endometritis group recovered. Three bitches in the acute endometritis group, two bitches in the acute metritis group and one bitch in the subacute metritis group died. The blood endotoxin level was determined to be quite high in the dead bitches. There was a difference in the levels of blood urea nitrogen (P<0.05), creatinine (P<0.01), and toxin (P<0.0001) between the dead and surviving bitches. In conclusion, the prognosis of pyometra may be recovered in bitches diagnosed with subacute endometritis associated with low levels of circulating endotoxin. The endotoxemic bitches that have blood endotoxin level above 0.96 EU/mL along with significantly increased alkaline phosphatase, blood urea nitrogen, and creatinine concentration would likely end up with a mortality outcome for acute endometritis, acute metritis, or subacute metritis. These parameters may serve as good prognostic markers in bitches with pyometra.

Keywords: *Bacteriology, Bitch, Histopathology, Prognosis, Pyometra*

Köpeklerde Endotoksik Pyometranın Klinik, Bakteriyolojik ve Histopatolojik Özellikleri

Öz

Bu çalışmanın amacı, pyometralı yirmi dört köpeğin uteruslarındaki histopatolojik değişikliklerin karşılaştırılmasıdır. Uterus lezyonları akut endometritis, subakut endometritis, akut metritis, subakut metritis olarak gruplandırıldı. Tam kan sayımı, kan biyokimyasal parametreler ve toksin seviyeleri belirlendi. Tüm gruplar arasındaki farklılık sadece alkalen fosfotaz seviyesinde önemliydi (P<0.01). Kan endotoksin seviyesi, akut ve subakut endometritis grupları (P<0.01) ile subakut endometritis ve akut metritis grupları (P<0.01) arasında farklıydı. Subakut endometritis grubundaki tüm köpekler iyileşti. Akut endometritis grubunda üç köpek, akut metritis grubunda iki köpek ve subakut metritis grubunda ise bir köpek öldü. Kan endotoksin seviyesinin ölen köpeklerde oldukça yüksek seviyede olduğu belirlendi. Ölen ve yaşayan köpekler arasında kan üre nitrojen (P<0.05), kreatinin (P<0.01) ve toksin (P<0.0001) seviyelerinin farklı olduğu görüldü. Sonuç olarak, dolaşımdaki endotoksinin düşük seviyesi ile ilişkili olarak subakut endometritis tanısı konulan köpeklerde pyometranın prognozunun iyileştirilebileceği düşünüldü. Yüksek seviyede alkalen fosfotaz, kan üre azotu ve kreatinin konsantrasyonu ile birlikte 0.96 EU/mL'nin üstündeki kan endotoksin düzeyine sahip endotoksemik köpeklerde akut endometritis, akut metritis veya subakut metritis sonucu ölüm görülmesinin muhtemel olabileceği kanısına varıldı. Bu parametreler, pyometralı köpeklerde iyi bir prognostik belirteçler olarak kullanılabilir.

Anahtar sözcükler: *Bakteriyoloji, Köpek, Histopatoloji, Prognoz, Pyometra*

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INTRODUCTION

Pyometra is a common inflammatory disease of the uterus and a hormone-related condition in adult bitches. It is characterized by genital and systemic illness with various clinical and pathological findings [1-3]. Prolonged or repeated progesterone exposure causes cystic endometrial hyperplasia, which is a thickening of the endometrial wall. When a bacterial infection and cystic endometrial hyperplasia occur in the uterus, pyometra becomes inevitable [1,4]. Most bacteria isolated from uterine content are gram negative bacteria that release lipopolysaccharide-structured endotoxin from their cell walls [1,3,5]. Multiple organ failure due to sepsis [6] and death may be observed in this disease [7-9]. Previous studies have shown that the mortality rate caused by pyometra is about 4-26% [7,9,10]. Renal dysfunction and hepatocellular damage caused by septicemia and/or diminished hepatic circulation and cellular hypoxia in a dehydrated bitch can lead to alteration in variety of serum biochemical parameters. In addition, suppression in bone marrow activity which leads to anemia and remarkably increased leukocytes can occur due to the toxin effect in bitches with pyometra related to endotoxemia [11,12]. Besides, the prognosis of pyometra may vary according to the absorption of bacterial toxin and the histomorphological alterations of the uterus [2,13-16]. Therefore, the determination of the blood endotoxin level and histopathological changes in the uterus are more important for estimating the prognosis of the disease in bitches with pyometra [2,14,17]. The prognosis and mortality prediction of pyometra remain the subject of research. The aim of this study is to evaluate the relationship between the blood endotoxin level and histopathological aspects according to the type of bacteria isolated from the uterine content and to investigate the risk status, prognosis, and possible death reasons in canine pyometra cases.

MATERIAL and METHODS

Animals

The present study was maintained in accordance with the directions of Guide for the Care and Use of Animal in Research. This research was performed in twenty-four bitches with pyometra from different breeds [Terrier (n=17), Collie (n=3), Cocker spaniel (n=1), Pekingese (n=1), Labrador retriever (n=1), Rottweiler (n=1)] and of varied ages (9.95±0.79 years; the range was 2-18 years) that were brought to the Gynecology Clinic of the Faculty of Veterinary Medicine, Ankara University. Pyometra was diagnosed by anamnesis and clinical examination including the evaluation of ultrasonographic findings (6-8 mHz with multifrequency linear and sector probes, B mode real time, Pie Medical, 100 Falco). In present study, the bitches were included with observation of at least one of sign such as vaginal discharge, anorexia, polyuria/polydipsia, lethargy, vomiting, dehydration, or weakness in the rear legs. Ovariohysterectomy was decided following the diagnosis of pyometra. Stabilizing the animals' conditions was attempted prior to surgery by giving intravenous fluid and antibiotics. Supportive therapy was maintained for at least seven days in the postoperative stage.

Serum Biochemical Parameters and Endotoxin Levels

Blood samples were preoperatively collected from the *vena cephalica antebrachii* into evacuated heparinized (Vacutest®; Arzergrande, Italy) tubes for complete blood-count-analysis and into plain tubes (Venoject®; Leuven, Belgium) for the determination of biochemical parameters and toxin levels. All samples were analyzed at The Laboratory of the Veterinary Faculty, Ankara University. The biochemical functions and blood toxin level were evaluated by centrifuging blood samples at 3000 rpm for 15 min and sera were stored at -80°C in a freezer until analysis. The serum biochemical parameters were evaluated using a chemistry analyzer (Reflotron® Plus, Roche, Istanbul): Asparateaminotransferase (AST), alanineaminotransferase (ALT), alkalinephosphatase (ALP), creatinine, and blood urea nitrogen (BUN) levels.

The blood endotoxin level was measured by utilizing an E-Toxate™ assay test kit (Sigma-Aldrich, Germany). The E-Toxate™ reagent working solution containing a Limulus Amebocyte Lysate (LAL) gel formulation assay was prepared according to the manufacturer's instructions.

Bacteriological Examination

Swab samples (Cultiplast®; LP Italiana SPA, Italy) for bacteriological examination were immediately taken under sterile conditions from uterine lumen following ovariohysterectomy. The all samples were cultured in an accredited laboratory (Accreditation No: AB-0031-T). The identification of isolates was performed with the conventional and Vitex automated method (Biomerieux, France). The isolates' susceptibility was assigned to post-operative antibiotic treatment. The minimum inhibitory concentrations were determined according to the standards of the National Committee of Clinical Laboratory Standards (NCCLS, 2000 and 2002), utilizing Mueller Hinton broth (Oxoid, UK).

The Evaluation of Histopathologic Aspect

After the ovariohysterectomy, the removed ovary and uterine tissues were submitted to the Laboratory of the Pathology Department at the Faculty of Veterinary, Ankara University for histopathological examination. The tissues were fixed in a 10% formaline buffer and embedded in paraffin. Paraffin blocks were sectioned at 5 µm, stained with Hematoxylin-Eosin (HδE), and examined under a light microscope. Microscopically, samples were classified as acute endometritis, subacute endometritis, acute metritis, and subacute metritis according to the inflammatory cell

infiltrations, hemorrhage in uterine layers, mucosal and vascular changes and glandular hyperplasia.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5.0 software (GraphPad, USA) and one-way ANOVA was performed to determine the differences between groups. *t-test* was used to compare two groups, and Fisher's exact test was used to evaluate the statistical significance of the difference between all blood parameters in the dead and surviving bitches. The results were expressed as mean±S.E.M. The level of significance for all statistical tests was $P<0.05$.

RESULTS

Gram negative bacteria were isolated in 18 cases and gram positive bacteria were isolated in five cases from uterine content; one case presented no growth. It was observed that 13 of the 23 cases were *Escherichia coli*. Open pyometra was recorded in 16 cases and closed pyometra was recorded in eight cases in the present study. The results of anamnesis, viability, and clinical findings were presented according to histopathological classification in *Table 1*. The differences between the groups for these results were not statistically significant.

The hematological and blood biochemical parameters of all groups are given in *Table 2*. Only the difference in ALP level was significant ($P<0.01$) between the groups. However, when the differences between the two groups were analyzed, ALT, ALP, BUN, creatinine, and lymphocyte values were found be significant *(Table 2)*. Although, there was no significant difference in the blood endotoxin level between the all groups ($P>0.05$), it was found significant for the difference between acute and subacute endometritis groups ($P<0.01$), and subacute endometritis and acute metritis groups ($P<0.05$). Besides, there was a difference in the levels of BUN ($P<0.05$), creatinine ($P<0.01$), and toxin ($P<0.0001$) between the dead and surviving bitches.

Uterine Pathological Alterations of the Cystic Endometrial Hyperplasia-Pyometra Complex

Macroscopic Findings

Generally, it was apparent that the subserosal veins associated with segmental expansion and thickening of the uterine mucosa were quite significant. Necrotic and/or hemorrhagic areas were observed in the uterine mucosa for seven cases and it was determined that six of these cases were dead after the ovariohysterectomy. In two of the dead cases in which Coagulase-positive Staphylococcus (CPS) was isolated, both necrotic and hemorrhagic areas were found. In four of the dead cases, gram negative bacteria were isolated and hemorrhagic areas were detected.

Microscopic Findings

Acute Endometritis: Microscopically, acute endometritis and glandular hyperplasia were observed in eight cases. Three bitches were dead after the ovariohysterectomy in this group. Gram negative bacteria (three *E. coli*, two *Pseudomonas aeruginosa)* were isolated in five cases and gram positive bacteria (two CPS, one Enterococcus) were isolated in three cases. Furthermore, CPS was isolated from another and *E. coli* was isolated from the uterine content in one of the dead bitches. The blood endotoxin level was significantly higher in the death cases in which *E. coli* was isolated *(Table 3)*. In this case, the degenerated surface epithelial cells, leukocytosis in the stratum vasculare, and cystic degeneration *(Fig. 1-a)* on the glandula uteri were markedly observed. In dead cases, isolated CPS, congestion, and hemorrhage on the stromal connective

Table 1. *The result of anamnesis, clinical examination, and viability findings according to groups*

Parameters and Features	Acute Endometritis (n=8)	Subacute Endometritis (n=5)	Acute Metritis (n=8)	Subacute Metritis (n=3)
MeanAge (year)	11.50±1.26	6.40±2.31	10.13±1.09	11.33±0.33
Breed	5 Terrier; 1 Cocker 1 Collie; 1 Pekignese	4 Terrier 1 Labrodor	5 Terrier; 2 Collie 1 Rotweiler	3 Terrier
Mean duration of disease (range day)	34.13±21.05 (7-180 days)	43.40±26.77 (20-210 days)	11.50±1.79 (7-21 days)	9.66±2.66 (7, 7 and 15 days)
Mean time of prostrus onset (day)	105.60±39.04	145.00±67.49	132.50±51.50	151.70±107.00
Previous litters	Yes (n=1) No (n=7)	Yes (n=2) No (n=3)	Yes (n=2) No (n=6)	No (n=3)
Cervix closure	Opened (n=7) Closed (n=1)	Opened (n=5)	Closed (n=8)	Opened (n=3)
Mean body temperature (°C)	39.79±0.07	39.42±0.03	39.24±0.1	39.44±0.05
Mean heart rate (beat/min)	123.50±0.43	125.44±0.12	128.32±0.23	124.65±0.14
Mean respiratory rate (breaths/min)	23.13±0.26	24.18±0.13	24.42±0.22	23.66±0.26
Viability	Survival (n=5) Death (n=3)	Survival (n=5)	Survival (n=6) Death (n=2)	Survival (n=2) Death (n=1)

Table 2. *The values of haematological and blood biochemical parameters between groups*

Cases	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	BUN (mg/dL)	Creatinin (mg/dL)	Erytrocyte (x10^{6}µl)	Leucocyte (x10^{3}µL)	Hct (%)	Band neut. (%)	Lymphocyte (%)
Acute Endometritis										
Case 1	70	10.7	249	18.59	1.18	6.5	60	35	27	1
Case 2*	70.4	18	212	121	3.07	5.5	61	29	14	4
Case 3	30.8	38.9	90	14.9	0.745	6.710	11.4	31	-	17
Case 4	57.6	41.2	149	27.33	0.889	3.26	54.5	25	9	5
Case 5	69	23.4	102	51.86	1.04	5.64	35	35	12	5
Case 6	36.6	21.9	135	18.83	0.529	4.1	44	25	5	4
Case 7*	61	60	130	42.05	2.8	5.2	28.2	34	27	25
Case 8*	108	90.5	399	134.11	0.959	7.25	59	30	11	2
Mean±SEM	62.93±8.37	38.08±9.32^{a}	183.30±36.19^{c}	53.58±16.79^{f,g}	1.40±0.34^{i,j}	5.52±0.47	44.14±6.37	30.50±1.43	13.13±3.39	7.87±3.00^{l}
Subacute Endometritis										
Case 1	45.6	61.1	170	14.43	0.625	6.64	16.4	37	30	9
Case 2	34.7	17.6	93.2	29.34	1.2	6.78	16.2	37	33	8
Case 3	72	29	121	9.70	0.85	4.7	90	28	8	7
Case 4	55.8	19	97	9.81	0.87	5.9	17.4	36	23	10
Case 5	61.3	27.5	226	18.92	0.866	3.55	60	24	19	11
Mean±SEM	53.88±6.41	30.84±7.89^{b}	141.40±25.19^{d}	16.44±3.64	0.88±0.09^{i,k}	5.51±0.61	40.00±15.06	32.40±2.69	22.60±4.41	9.00±0.70^{l,m}
Acute Metritis										
Case 1*	34.9	18.8	255	85.98	1.45	4.9	55.3	22	18	**9**
Case 2	33.7	20.4	458	20.04	1.29	5.02	26.1	35	13	7
Case 3	89.3	23.5	208	42.99	1.9	5.0	5.0	31	7	3
Case 4	73	10.1	204	19.62	0.865	5.9	29.8	31	27	4
Case 5	64.7	23.5	212	19.62	0.856	3.66	60.0	24	19	11
Case 6*	70.2	12.2	305	54.67	3.3	4.8	47.4	27	18	7
Case 7	55.6	27.4	633	18.64	0.871	7.6	16.8	40	4	10
Case 8	85.4	19.8	256	41.12	1.8	5.4	82.5	21	38	-
Mean±SEM	63.35±7.38	19.46±2.05^{a,b}	316.40±53.97^{c,d,e}	37.84±8.43^{h}	1.54±0.29^{k}	5.28±0.39	40.36±9.01	28.88±2.34	18.00±3.83	6.37±1.33
Subacute Metritis										
Case 1	45.9	5	96	11.02	0.826	4.1	63.2	24	22	4
Case 2*	55	12.8	20	140.18	8.1	5.92	58.3	21	24	7
Case 3	56.8	19.8	149	40.18	2.34	4.95	13	36	4	18
Mean±SEM	52.57±3.37	12.53±4.27	88.33±37.43^{e}	63.79±39.11^{g,h}	3.75±2.21^{j}	4.99±0.52	44.83±15.98	27.00±4.58	16.67±6.36	9.66±4.25^{m}

** dead cases*
There is a statistical importance between the values shown with different letters in the same column (b. k. i. k. l. m P< 0.01) (d. e. h. P<0.05) (a. c. f. g. j P<0.001)

tissue and degenerative hyperplasia and atrophy on the glandula uteri were determined *(Table 3)*.

Subacute Endometritis: Subacute endometritis was microscopically detected in five cases. *E. coli* (n=2), *K. oxytoca* (n=1) and *ß-hemolytic streptococcus* (n=2) were isolated in this group. All of these bitches recovered after the ovariohysterectomy. Glandular hyperplasia was found in the uterus of a case in which *E. coli* and *K. oxytoca* were isolated *(Fig.1-b)*. Squamous metaplasia on the surface epithelial cells was determined in the case of isolated *K. oxytoca*. An atrophic uterine gland was detected in two cases of isolated *ß-hemolytic streptococcus*. Squamous metaplasia was observed on the surface epithelial cells in one of these cases *(Table 4)*.

Acute Metritis: Acute metritis was determined in eight cases. Two bitches died after the ovariohysterectomy in the group. While no bacterial growth was observed in one case, gram negative bacteria (five of *E. coli*, one of *Ps. aeruginosa*, and one of *K. pneumonia*) were isolated in other cases. Papillary hyperplasia and cyst-like honeycomb were detected in the case with no bacteria and with isolated *K. pneumonia*. There was inflammation in both

Table 3. *The histopathological view of cases with acute endometritis*

Cases	Surface Epithelial Cells	Inflammation		T. Muscularis	Str. Vasculare	Stromal Connective Tissue	Gl.Uteri	Isolated Bacteria	Time to Onset of the Disease (day)	Toxin Level (EU/mL)	Cervix	Viability
		Endomet.	Myomet.									
1	Normal	+	-	Hyperemia	Leukocytosis, swelling in endothelial cells	-	Large cysts	E coli	20	0.24	Opened	Survival
2	Degenerated	++	-	Hyperemia	Leukocytosis	Congestion	Cystic degenaration	E coli	30	3.84	Opened	Death
3	Normal	-	-	Congestion	-	Congestion, Hemorrhage	Uniform	E coli	7	0.48	Opened	Survival
4	Normal	+	-	Hyperemia	-	Hemorrhage	Papillary hyperplasia, Large cysts	Ps aeruginosa	180	0.48	Opened	Survival
5	Degenerated	+	+	Hyperemia	-	Oedemma	Large cysts	Ps aeruginosa	7	0.96	Closed	Survival
6	-	-	-	-	-	-	Papillary hyperplasia, Large cysts	Enterococ	15	-	Opened	Survival
7	-	+	-	-	-	Oedemma	Atrophic	CPS	7	-	Opened	Death
8	Degenerated	+	-	-	-	Oedemma, Congestion, Hemorrhage	Degenerated hyperplasia	CPS	7	-	Opened	Death

Endomet.: Endometrium; Myomet.: Myometrium; T. muscularis: Tunica muscularis; Str. vasculare: Stratum vasculare; Gl. uteri: Glandula uteri; CPS: Coagulase positive Staphylococ

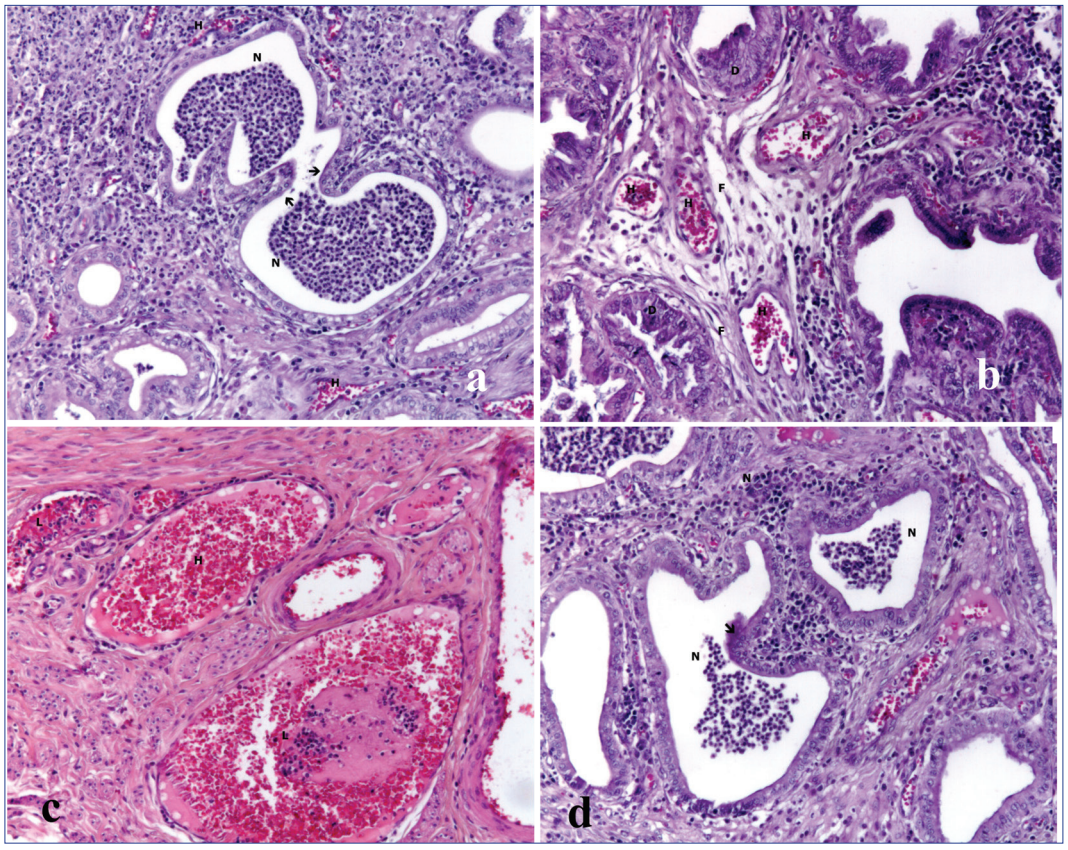

Fig 1. The view of histopathological changes in uterine tissue. **(a)** Acute endometritis; neutrophile leucocyte in lamina propria and tunica muscularis (N) and few mononuclear cell infiltration, papillar cystic dilatations in glands *(arrows)* and hyperemia (H), **(b)** Subacute endometritis; hyperplasie in gland epithelial cells (D), connective tissue in lamina propria (F) and other mononuclear cell infiltrations, hyperemia (H), **(c)** Acute metritis; leukostasis in tunica muscularis veins (L) and hyperemia (H) **(d)** Subacute metritis; mononuclear inflammatory cells aand connective tissue in lamina propria with neutrophile leukocyte infiltration in gland lumen (N), papillar hyperplasie *(arrow)* and cystic dilatations in glands and hyperemia (H), HE, x100

Table 4. *The histopathological view of cases with subacute endometritis*

Cases	Surface Epithelial Cells	Inflammation		T. Muscularis	Str. Vasculare	Stromal Connective Tissue	Gl.Uteri	Isolated Bacteria	Time to Onset of the Disease (day)	Toxin Level (EU/mL)	Cervix	Viability
		Endomet.	Myomet.									
1	Squamous metaplasia in some area	+	-	Hyperemia	-	Oedemma, Congestion, Hemorrhage, fibroblast proliferation	Large cysts	*K oxytoca*	210	0.24	Opened	Survival
2	Degenerated	+	-	Hyperemia	-	Oedemma, Congestion	Large cysts	*E coli*	20	0.48	Opened	Survival
3	Degenerated	+	+	Hyperemia	Leukocytosis	Oedemma, Congestion	Hyperplasia, Large cysts	*E coli*	20	0.48	Opened	Survival
4	Normal	++	-	Hyperemia, Intense hemorrhage	-	Congestion, Intense hemorrhage	Intense atrophic	β HemolyticStrep	150	-	Opened	Survival
5	Metaplasia	-	-	-	-	Hemorrhage	Atrophic	β HemolyticStrep	20	-	Opened	Survival

Endomet.: Endometrium; Myomet.: Myometrium; T. muscularis: Tunica muscularis; Str. vasculare: Stratum vasculare; Gl. uteri: Glandula uteri; β HemolyticStrep: ß hemolytic Streptococus

Table 5. *The histopathological view of cases with acute metritis*

Cases	Surface Epithelial Cells	Inflammation		T. Muscularis	Str.Vasculare	Stromal Connective Tissue	Gl.Uteri	Isolated Bacteria	Time to Onset of the Disease (day)	Toxin Level (EU/mL)	Cervix	Viability
		Endomet.	Myomet.									
1	Squamous metaplasia	+	+	Hemorrhage	Leukocytosis, swelling in endothelial cells	Congestion, Intense hemorrhage	Irregular stroma, Small cysts	*E coli*	10	1.92	Closed	Death
2	Degenerated	+	-	-	Hyperemia	Oedemma, Congestion, Hemorrhage	Cystic degenaration	*E coli*	7	0.96	Closed	Survival
3	Degenerated	+	-	-	Leukocytosis, swelling in endothelial cells	Oedemma, Congestion, Hemorrhage	Papillary hyperplasia, Large cysts	*E coli*	15	0.96	Closed	Survival
4	Degenerated	+	-	Hyperemia	Leukocytosis	Oedemma, Congestion, Hemorrhage, Fibroblast proliferation	Irregular stroma, Small cysts	*E coli*	10	0.96	Closed	Survival
5	Degenerated	++	-	Hyperemia	-	Fibroblast proliferation	Papillary hyperplasia, Large cysts	*E coli*	21	0.48	Closed	Survival
6	Squamous metaplasia	+	+	Oedemma	Leukocytosis, Hyperemia	Oedemma, Congestion	Large cyst	*Ps aeruginosa*	7	3.84	Closed	Death
7	Degenerated	+	-	Hyperemia	Leukocytosis, swelling in endothelial cells	Congestion, Hemorrhage	Papillary hyperplasia, Cysts-like honeycomb	*K pneumonia*	7	0.48	Closed	Survival
8	Degenerated	+	-	Hyperemia	Leukocytosis	Oedemma, Congestion	Papillary hyperplasia, Cysts-like honeycomb	No growth bacteria	15	-	Closed	Survival

Endomet.: Endometrium; Myomet.: Myometrium; T. muscularis: Tunica muscularis; Str. vasculare: Stratum vasculare; Gl. uteri: Glandula uteri

the endometrium and myometrium, and squamous metaplasia on the surface epithelial cells was found in dead bitches compared to recovered bitches. In the dead cases in which *E. coli* was isolated, the blood endotoxin level was 1.92 EU/mL. Intensive hemorrhage on the stromal connective tissue was discovered in this case *(Fig. 1-c)* and the blood endotoxin level in the dead case with isolated *Ps. aeruginosa* was 3.84 EU/mL; unlike the other cases, edema was seen in the tunica muscularis *(Table 5)*.

Subacute Metritis: Subacute metritis was seen in three cases, one case died following the operation. *E. coli* was isolated in all three cases, and inflammation was found in both the endometrium and myometrium in these cases.

Table 6. *The histopathological view of cases with subacute endometritis*

Cases	Surface Epithelial Cells	Inflammation		T. Muscularis	Str. Vasculare	Stromal Connective Tissue	Gl. Uteri	Isolated Bacteria	Time to Onset of the Disease (day)	Toxin Level (EU/mL)	Cervix	Viability
		Endomet.	Myomet.									
1	Normal	+	+	Oedemma, Hyperemia	Leukocytosis, swelling in endothelial cells	-	Hyperplasia, Large cysts	*E coli*	7	0.48	Opened	Survival
2	Degenerated	+++	+++	Oedemma, Hemorrhage	Leukocytosis, Hyperemia	Congestion	Large cysts lining to papillary extension	*E coli*	15	0.96	Opened	Death
3	Normal	+	+	-	-	Hemorrhage, Congestion	Uniform	*E coli*	7	0.96	Opened	Survival

Endomet.: Endometrium; Myomet.: Myometrium; T. muscularis: Tunica muscularis; Str. vasculare: Stratum vasculare; Gl. uteri: Glandula uteri

The inflammation was severe in the dead bitch *(Fig. 1-d)* and the degeneration of the surface epithelial cells was established in this bitch *(Table 6)*.

DISCUSSION

Pyometra, which is also known as cystic endometrial hyperplasia-pyometra complex, is a uterine bacterial infection associated with the thickening of the endometrium due to an increase in the size and number of endometrial glands [4,10,17]. If pyometra is allowed to continue untreated, death may occur due to bacterial toxic shock and multiple organ failure [4,9,11]. However, bacterial endotoxins damage the vascular endothelial structure and cause a prothrombin-antithrombin imbalance; this situation could lead to disseminated intravascular coagulation (DIC) and clinical findings may be severe in bitches with endotoxic pyometra. The prognosis of the disease is more important and the surgical approach may carry a high risk of death [18-20]. It was determined that gram negative bacteria (three *E. coli* and one *Ps. aeruginosa*) were isolated from four out of the six bitches that died. We conceived that the death could be observed due to DIC or/and multiple organ dysfunction in these bitches with poor prognosis.

The disease is generally caused by a Gram negative bacteria, typically *E. coli* [21], but other bacterial species such as staphylococci, streptococci, *Enterococcus* spp., *Klebsiella* spp. and *Ps. aeruginosa*, *Proteus* spp., *Pasteurella* spp. could also been detected [11,22,23]. It has been established that if *E. coli* migrates to the uterus, it binds with higher affinity to the endometrium and myometrium layers and constitutes a resistance to the uterus' defense mechanisms such as lactoferrin and mucin-1 [24-27]. As reported in previous studies [4,11,23,28], gram negative bacteria (75%) such as *E. coli*, Klebsiella spp. and *Ps. aeruginosa* were dominantly isolated and *E. coli* (54.16%) was the most isolated bacteria in this study.

It is well known that changes in levels of liver and kidney enzymes could be caused by the effects of gram negative bacterial endotoxin [2,11,29,30]. Furthermore, bacterial endotoxins induce a marked increase in the number of circulating polymorphonuclear phagocytes (regenerative left shift). In addition, anemia could be observed due to the bone marrow suppression by bacterial endotoxins [2,24,30-32]. In the present study, the changes in the different degrees were observed in the blood biochemical and hematological parameters in bitches with isolated gram negative bacteria. The levels of ALP, BUN, and creatinine showed a positive correlation (r=0.87) with the blood endotoxin level. The increased levels of ALP, renal deficiency, and high toxin levels in the bitches with isolated gram negative bacteria were associated with each other in pyometra, as indicated in previous studies [2,33,34]. The bitches in which significant deviations in the levels of BUN ($P<0.01$), creatinine ($P<0.01$) and toxin ($P<0.0001$) were observed showed severe clinical signs and poor prognosis, and were dead within two days following the ovariohysterectomy. We thought that the high toxin amount in these bacteria lead to damage in the liver and kidney; in particular, irreversible acute renal failure caused death, as reported in previous studies [4,9,11,35,36].

A uterine content swab obtained from one bitch in our study was bacteriologically sterile. Bacteria caused by this disease may have been killed by uterine defense mechanisms or antibiotic treatment [37]. The leucocyte count was found to be significantly elevated (82.5 x 103 µL) in this bitch. In previous studies, neutrophils consisting of high concentration lactoferrin, which has a critical role in the uterine defense mechanism, infiltrated the uterus and glandular epithelial cells might express this protein to kill bacteria or inhibit bacterial growth [24,38-40]. In the present study, CPS was isolated in two bitches. Severe clinical symptoms including increased blood biochemical and hematological parameters were observed, and the time to onset of the disease was seven days in these bitches. These cases were dead within two days following the ovariohysterectomy. Staphylococci species can cause a variety of life-threatening infections such as sepsis and toxic shock syndrome. These species released harmful proteins and enzymes, which can cause tissue and

organ damage [41,42]. We thought that these bitches were irreversibly affected by coagulase-positive staphylococci in a short time period and death was observed.

Beta hemolytic streptococci, like staphylococci, can cause life-threatening acute infections and deaths due to their secreted virulence factors such as streptolysin. In addition, these organisms stimulate the production and activities of superantigens. These superantigens are important in the development of toxic shock syndrome [42,43]. In this study, beta hemolytic streptococci were isolated in two bitches; clinical and laboratory findings were not severe, and these cases survived after the operation. We noted that systemic antibiotics had been occasionally administered to these bitches with open cervix pyometra at another clinic, but the bitches had shown no improvement. It was considered that antibiotics could slow the disease's progression. Enterococcal infections have long been associated with a higher risk of treatment failure because of their resistance to many antibiotics (penicillin by beta-lactamases, chloramphenicol, tetracyclines, rifampin, fluoroquinolones, and aminoglycosides) and mortality [44]. In the present study, Enterococcus was isolated in one case. Blood biochemical parameters within the reference values and changes in the mid-level hematological parameters were determined. The clinical symptoms were not severe and this case recovered after the operation. It is thought that this bitch survived due to appropriate antibiotic administration and there were no severe clinical findings.

In conclusion, the results of the present study revealed that the prognosis of the disease may improve in bitches diagnosed with subacute endometritis associated with low levels of blood endotoxin. However, for acute endometritis, acute metritis, and subacute metritis, the endotoxemic bitches with a blood endotoxin level above 0.96 EU/mL and significantly increased ALP, BUN, and creatinine concentration would likely end up with mortality outcome following ovariohysterectomy. These parameters may serve as good prognostic markers in bitches with pyometra.

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