Investigation on the Antiendotoxic Effect of the Combination of Polymyxin E and Ampicillin in Dogs with Endotoxic Pyometra

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Summary

The aim of this study was to determine the incidence and levels of the gram negative bacterial endotoxin, and to investigate the effects of antiendotoxemic treatment along with operation. Thirty bitches with pyometra were used in this study. The bitches were randomly allocated into two groups as Group I (treated with polymyxin E-ampisillin) and Group II (treated with ampisillin). Samples for bacteriological examination were taken from vagina, vesica urinaria and uterus of all bitches. Blood samples were collected to evaluate biochemical, haematological tests and serum toxine levels before the operation and on 7 days after operation. Gelation was carried out to determine the gram negative bacterial endotoxin level with E-toxate test kit in accordance with gelation system. Gram negative bacteria were isolated 66% in Group I and 80% Group II. *Escherichia coli* were dominantly (63.4%) isolated from the uteri of 30 bitches with pyometra. Before the operation, serum toxin levels were found in Group I (n=10) 1.2±0.4 EU/ml and in Group II (n=12) 0.9±0.2 EU/ml. Five bitches in Group I and 3 bitches in Group II died on the third day after operation. Survival rate was a positive correlation with toxin levels (r=0.727; P<0.01) and BUN (r=0.539; P<0.01), creatinin (r=0.504, P<0.05) before operation in recovered and dead bitches. Serum toxin levels were not different between Group I (n=6; 0.14±0.02 EU/ml) and Group II (n=11; 0.19±0.01 EU/ml) 7 days after operation. Difference of toxin levels in both groups were different (P<0.05) between the preoperation period and the 7th day after operation. Before the operation and on the 7th day after the operation, in Group I difference between band neutrophil count and in Group II ALT, ALP, BUN, creatinin, RBC, WBC, Hct, band neutrophil and lenphocyte value were found significant (P<0.05). As a result, there was a positive correlation between toxin levels and BUN-creatinin values. Also, the cases that have the increased BUN and creatinin levels along with high toxin levels were trended towards to low survival rate. After operation, in cases that performed with ampicillin were acquired more effective results contrary to the hipothesis according to polymyxin E+ampicillin combination in bitches with pyometra.

Keywords: *Antiendotoxemic treatment, Bitch, Endotoxemia, Pyometra*

Endotoksik Pyometralı Köpeklerde Polimiksin E - Ampisilin Kombinasyonunun Antiendotoksik Etkisinin Araştırılması

Özet

Bu çalışmanın amacı, pyometra olgularında gram negatif bakteriyel endotoksinin görülme sıklığı ve düzeyinin belirlenmesi ve operasyonla birlikte antiendotoksemik tedavi etkinliğinin araştırılmasıdır. Çalışma materyalini pyometra tanısı konulan 30 dişi köpek oluşturdu. Pyometralı köpekler rastgele Grup I (polimiksin E-ampisilin tedavisi olanlar) ve Grup II (ampisilin tedavisi olanlar) olmak üzere iki gruba ayrıldı. Her olgunun uterus, vagina ve idrar kesesinden mikrobiyolojik svap örnekleri alındı. Operasyon öncesinde ve sonrası 7. günde tüm olguların kan endotoksin, serum biyokimya ve hematolojik parametreleri değerlendirildi. Endotoksin düzeyini belirlemek amacıyla jelasyon yöntemine göre çalışan E-toxate® test kitinden yararlanıldı. Gram negatif bakteriler Grup I'de %66, Grup II'de %80 oranında izole edildi. Pyometralı 30 köpeğin uterusundan baskın olarak (%63.4) *Escherichia coli* izole edildiği saptandı. Operasyon öncesi Grup I (n=10)'e ait olgularda serum toksin düzeyinin 1.2±0.4 EU/ml, Grup II (n=12)'de ise 0.9±0.2 EU/ml olduğu belirlendi. Operasyondan sonraki 3. günde Grup I'de 5 olgunun, Grup II'de 3 olgunun öldüğü görüldü. Yaşayan ve ölen hayvanlarda, ölüm riski ile operasyon öncesi BUN (r=0.539, P<0.01), kreatinin seviyesi (r=0.504, P<0.05), toksin düzeyi (r=0.727, P<0.01) arasında pozitif korelasyon belirlendi. Operasyon sonrası 7. günde Grup I (n=6; 0.14±0.02 EU/ml) ve Grup II (n=11; 0.19±0.01 EU/ml)'de belirlenen toksin düzeylerinin operasyon öncesi değerlerine göre düştüğü (P<0.05) saptandı. Grup I ve Grup II'de operasyon sonrası toksin düzeylerindeki azalmanın önemli olmadığı (P>0.05) görüldü. Operasyon öncesinde ve sonrası 7. günde Grup I'de band nötrofil sayısı arasındaki farklılık önemli bulunurken, Grup II'de ALT, ALP, BUN, kreatinin, alyuvar, akyuvar, hematokrit, band nötrofil ve lenfosit oranı arasındaki farklılığın önemli olduğu (P<0.05) saptandı. Sonuç olarak toksin seviyesi ile BUN ve kreatinin düzeyleri arasında pozitif korelasyon olduğu ve bu değerleri yüksek bulunan olguların yaşayabilirlik oranının düşük olduğu, operasyonla birlikte ampisilin uygulamasının polimiksin E+ampisilin kombinasyonuna göre hipotezin aksine köpeklerde pyometra olgularında daha etkili olduğu kanısına varıldı.

Anahtar sözcükler: *Antiendotoksemik tedavi, Endotoksemi, Köpek, Pyometra*

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INTRODUCTION

Pyometra is a common diestral disease in adult intact bitches characterised by genital and systemic illness with variety of clinical symptoms and inflammatoric uterus accumulation [1-4]. Common bacteria isolated from pyometra infections in bitches are gram negative bacteria such as *Escherichia coli, Klebsiella spp., Pasteurella, Proteus, Pseudomonas spp.,* which releases lipopolysaccharide structured endotoxine from their cell wall [1,5-8]. In the case that infection with predominantly gram-negative bacteria such as *Escherichia coli* are isolated, endotoxemia has been demonstrated in bitches with pyometra [6,9]. According to the main theories, this bacteria rise as ascending bacterial infections from the host's vaginal/intestinal flora or as descending from urinary tract infection [8,10-12]. Renal dysfunction and hepatocellular damage caused by septicemia and/or diminished hepatic circulation and cellular hypoxia in the dehydrated bitch lead to variety of serum biochemical parameters. Also the suppression of bone marrow activity by toxemia is caused anemia and in cases of pyometra are remarkably increase leucocyte [3,7,13,14]. In recent years, blood endotoxin levels have been reported in bovine gangrenous mammilitis and equine colic, but little attention has been paid to blood endotoxin levels in small animals [6]. In case of pyometra, to measure the concentration of endotoxin in blood is considerable. It is therefore necessary to initiate effective treatment in the early stage of the disease for bitches with high blood endtoxin concentration [2,6]. The aim of this study was to determine the incidence and levels of the gram negative bacterial entoxin; to investigate the effects of antiendotoxemic treatment along with operation.

MATERIAL and METHODS

The present study was performed in 30 privately owned bitches with pyometra from different breeds and age which were brought to the Gynaecology Clinic of the Veterinary Faculty of Ankara University from July 2005 to January 2006. The ages of the animals ranged from 2 to 18 years. Diagnosis of pyometra was based on case history, clinical examination including ultrasonography (6-8 mHz with multifrequency linear and sectorial probes, B mode real time ultrasonographic device, Pie Medical, 100 Falco). Ovariohysterectomy was implemented to all bitches under general anesthesia protocols. After the operation, the bitches were randomly allocated into two groups. In Group I (n=15), an antiendotoxemic agent polymyxin E-ampicillin (Colicillin®, Egevet, Izmir) was applied 1 ml/10 kg IM for four days. In Group II, ampicillin (Ampisina® 250-500 mg, Mustafa Nevzat, Istanbul) was given four days long via intramuscular way. Samples for bacteriological examination were taken under sterile conditions from vagina, vesica urinaria and uterus. Uterine swabes were obtained by insision; urine samples were collected by cystosynthesis method; vaginal specimens were taken by sterile cotton swabes via speculum. Sterile swaps (Cultiplast®; LP Italiana SPA, Italy) were used for bacteriological isolation where the samples were sent to an accredite laboratory (Accreditation No: AB-0031-T) and Conventional and Vitex automatic method (Biomerieux, France) were used for identification of isolates. Blood samples for haematology, biochemical panels and serum endotoxin determinations were obtained immediately in preoperative period and the seventh day of postoperative period in all the bitches. Blood samples were collected from the vena cephalica antebrachii into evacuated heparinised (Vacutest®; Arzergrande, Italy) tubes for complete blood-count-analysis in the Department of Physiology, Faculty of Veterinary Medicine, Ankara University. Also, for determination of biochemical parameters (AST, ALT, ALP, BUN ve creatinine) in Laboratory of the Veterinary Faculty, plain tubes (Venoject®; Leuven, Belgium) were used for sample collection. Commercial E-toxate kit (Sigma-Aldrich, Germany), working with gelation procedure, was used for determination of blood endotoxin levels.

The statistical analyses were performed by SPSS 14.01 (serial:9869264) for the evaluation of toxin values, survival rates and serum biochemical, haematological parameters between groups. Differences were considered to be significant if P-values were lower than 0.05. The results are expressed as the mean and standart error. Wilcoxon was performed for controling the significance of difference between groups. According to the controling the significance of difference in blood parameters between groups and each group; Wilcoxon and Mann Whitney U tests was performed, respectively. Pearson's correlation test was used for the evaluation of blood parameters' between preoperative and postoperative process.

RESULTS

In Group I and Group II, the breed distribution was identified as Terrier, Doberman, Rotweiler, Kangal, Collie, Pekingese, Labrador, Poodle, Cocker. Terrier breeds were dominantly (63.3%) seen among these breeds. In Group I, mean age of bitches with pyometra was identified as 9.6±0.9 years (2-15 years old), in Group II 10.9±0.8 years (5-18 years old) (P>0.05).

Microbiological Results

In all cases, 63 of 90 (70%) swap samples which have taken from uterus, vagina and vesica urinaria, micro-

organisms were isolated and it was observed that 40 (63.4%) of these samples were *Escherichia coli*. The dispersion of these microorganisms isolated from uterus, vagina and vesica urinaria according to groups is shown in *Table 1*. In Group I, same bacteria was isolated from uterus and vagina in 13 cases (43.3%), uterus and vesica urinaria in 4 cases (13.3%) and uterus, vagina and vesica urinaria in 10 cases (33.3%). In Group II, the bacteria was isolated from uterus and vagina in 8 cases (53.3%), uterus and vesica urinaria in 2 cases (13.3%), uterus, vagina and vesica urinaria in 4 cases (26.6%).

reference levels; and it was seen no alterations (P>0.05) in the variations of heamotologic parameters between the Groups *(Table 2)*. Considering the relation between toxin levels and heamotologic parameters, it was observed that the variations in these cases were similar.

Viability findings: In this study, 22 of the 30 bitches (73.3%) have survived after the operation, 8 bitches (26.6%) died 3 days after operation. While 5 of death cases (62.5%) were identified in Group I, 3 of these cases (37.5%) were in Group II. There were positive correlations

Table 1. Distribution of microorganisms in groups that isolated from uterus, vagina and vesica urinaria
Tablo 1. Uterus, vagina ve vesica urinaria'dan izole edilen mikroorganizmaların gruplara göre dağılımı

Group	Isolated Bacteria	Uterus	Vagina	V. Urinaria
Group I (n=15)	*E. coli*	7 (46.6%)	4 (26.6%)	4 (26.6%)
	K. pneumonia	1 (6.6%)	1 (6.6%)	1 (6.6%)
	K. oxytoca	1(6.6%)	-	1 (6.6%)
	Ps. aeruginosa	1(6.6%)	-	1 (6.6%)
	ß-Haemolytic Streptococci	1 (6.6%)	1 (6.6%)	1 (6.6%)
	Enterococcus	1 (6.6%)	1 (6.6%)	-
	Mix infection (E. coli and ß Haem Strep.)	1 (6.6%)	-	-
	Total	**13 (86.6%)**	**7 (46.6%)**	**8 (53.3%)**
	Negative isolation	2 (13.3%)	8 (53.3%)	7 (46.6%)
Group II (n=15)	*E. coli*	10 (66.6%)	9 (60%)	6 (40%)
	Ps. aeruginosa	2 (13.3%)	2 (13.3%)	1 (6.6%)
	Coagulaz positive Staphylococci	2 (13.3%)	2 (13.3%)	1 (6.6%)
	Total	**14 (93.3%)**	**13 (86.6%)**	**8 (53.3%)**
	Negative isolation	1 (6.6%)	2 (13.3%)	7 (46.6%)

Preoperative Laboratory Parameter Results

Blood endotoxin level: In pyometra cases, considering the relation between serum toxin levels with clinical findings and the outbreak of the disease, according to the microbiological cultures 10 cases (66%) in Group I and 12 cases (80%) in Group II was determined as gram negative microorganisms and developing endotoxemia. Serum toxin levels were established as for Group I 1.2±0.4 EU/ml (0.24-3.84 EU/ml) and for Group II 0.9±0.2 EU/ml (0.48-3.84 EU/ml).

Serum biochemical parameters: The pyometra cases included in this study had an increase in ALP, BUN and creatinine values, however ALT was proximal to basal level and in both groups, these findings were similar. Preoperative serum biochemical parameters for Group I and Group II are shown in *Table 2*. Considering the serum biochemical parameters of toxin level determined cases, it was determined that in cases with high toxin levels, the reference levels of ALP (158±78.5 IU/L), BUN (62.7±44.3 mg/dl) and creatinine (2.5±2.2 mg/dl) were considerably higher.

Haematologic parameters: In all of cases, red blood cells, haematocrit and lymphocyte values were observed below the reference level; while the number of band neutrophils and leucocytes were detected over the

between survival rate and toxin level (r=0.727, P<0.01), BUN (r=0.539, P<0.01) and with creatinine level (r=0.504, P<0.05). Also toxin level showed a positive correlation-way with BUN level (r=0.496, P<0.01) and creatinine (r=0.527, P<0.01). However, a strong positive directional correlation (r=0.777) was found between BUN and creatinine levels of death and survival cases.

Postoperative Laboratory Parameter Findings

Blood endotoxin levels: After infection source was removed by ovariohysterectomie, antiendotoxemic treatment applied Group I and ampicillin treatment applied Group II cases for blood endotoxin level designated postoperative on day 7th, as well as between both groups were compared with data before the operation to determine the effectiveness of the treatment. Blood endotoxin levels in 6 out of 10 survived cases for Group I (0.14±0.02 EU/ml), 11 cases in Group II (0.19±0.01 EU/ml) were detected 7 days after operation. For these values while the differences between the groups were not significant, the internal differences the groups were important (P<0.05). However, the diversity of improvement in groups after the operation was not significant in dogs experiencing clinical symptoms (P>0.05).

Serum biochemical parameters: There was difference between the values of BUN (P<0.05) between Group

I (n=10) and Group II (n=12) 7 days after operation. Polymyxin E-ampicillin combination applied to a group belonging cases serum biochemical parameters was not different on preoperation and 7 days postoperation (P>0.05). Ampicillin applied belongs to the group in the AST value except for all the parameters of the difference between the significant (P<0.05) was determined *(Table 2)*.

cases of Grup II were proceeded with endotoxemia. This situation has confirmed that, *E. coli* migrates to uterus, connects to endometrium and myometium layers which the bacteria shows affinity, and shows resistance to uterus' defence mechanisms such as lactoferrin and mucin-1 [8,18]. Besides, for 3 cases included in this study, we considered that the reason for not identifying any

Table 2. *Statistical significancy, mean and standart error values of serum biochemical and haematological parameters in preoperative and postoperative period in groups*

Tablo 2. *Operasyon öncesi ve sonrası serum biyokimyasal ve hematolojik parametrelerin gruplara göre ortalama/standart hata değerleri ile istatistiki önemleri*

Parameters/ Reference Ranges	Group I		Group II	
	Pre-operative (Min-Max/X±Sx) (n=15)	Post-operative (Min-Max/X±Sx) (n=10)	Pre-operative (Min-Max/X±Sx) (n=15)	Post-operative (Min-Max/X±Sx) (n=12)
AST (U/L) (≤25 U/L)	25.5-96.1 58.06±5.86	22.1-84.8 42.93±7.15	33.7-108 64.95±5.14	30.2-71.5 45.25±3.74
ALT (U/L) (≤55 U/L)	10.7-61.1 25.01±3.3	13.2-133 51.81±10	10.1-90.5 29.48±5.3 *	26.8-94.5 37.93±5.43 *
ALP (U/L) (≤110 U/L)	85-895 239.55±59.02	20-499 176.92±43.06	54.6-458 189.52±29.07 *	20-402 131.67±28.45 *
BUN (mg/dl) (10-28 mg/dl)	9.81-140.18 48.16±14.94	9.5-22.7 16.46±1.37	9.7-134.11 43.17±9.35 *	16.8-31.02 22.17±1.49 *
Creatinine (mg/dl) (≤1.5 mg/dl)	0.529-8.10 1.58±0.51	0.6-1.2 0.8±0.6	0.832-3.07 1.4±0.1*	0.5-1.1 0.82±0.06 *
RBC (10^6 µl) (6.1-8.7x10^6 µl)	3.9-8.0 5.82±3.75	3.1-7.9 5.89±4.75	3.25-7.25 5.33±2.66 *	1.02-7.9 5.49±5.73 *
WBC (10^3 µl) (6-16x10^3 µl)	7.1-63.2 31.91±5.35	6.4-46.5 25.13±4.52	5-90 42.75±7.2 *	2.7-57.2 18.7±4.48 *
Hct (%) (43-59%)	21-45 30.67±1.97	17-45 32.4±2.3	21-40 31.33±1.36 *	12-42 31.75±2.39 *
Band neutrophile (%) (0-3%)	4-37 19.27±2.81 *	0-26 7.6±2.4 *	7-38 17.67±2.84 *	2-8 4.17±0.5 *
Seg. neutrophile (%) (60-77%)	53-84 68.07±2.43	53-89 73±3.3	53-84 62.87±3.3	68-88 80.17±1.94
Lymphocyte (%) (12-30%)	1-17 9.47±1.32	2-16 11.8±1.4	0-27 10.13±2.21 *	4-12 10.92±1.44 *

* in the same line denote significant differences between means (P<0.05)

Haematologic Parameters: The difference between the groups of segmented neutrophil rate was found significant (P<0.05) 7 days after operation. As a haematological parameter while ratio of the difference between within the polymyxin E-ampicillin group cases, the band neutrophil (P<0.05) were detected be significant, within the survived ampicillin group cases, the ratio of difference between the erythrocyte and leukocyte count and haematocrit, band neutrophil, and lymphocyte (P<0.05) revealed as significant.

DISCUSSION

In current study, parallel to other researchers [6,15-17], gram negative bacteria (73.3%), especially *E. coli* was observed dominate and 10 cases of Group I, and 12

bacteria, the fagositic activities of leucocytes migrate to uterus as it was not spesified in previous studies [19,20]. However, as indicated in the literature [8,10-12] isolating the same bacteria species in 13 cases from vagina and uterus; in 6 cases from uterus and the vesica urinaria, in 8 cases from uterus, vagina and vesica urinaria from both groups made us think that bacteria cause pyometra can come as ascendingly to uterus from skin or intestine orginated from vaginal flora or descendingly from the urinary system working such as an individual bacterial reservoir either.

Despite pyometra can appear in every age and breed, it approximately appears 7 years and older dogs and varies in geographic regional breeds [21]. As indicated in the literature, in this study, 86.6% of pyometra cases are appeared to be in 7 years and above ages, [19,22-24]

confirms pyometra progresses with age, when the uterus' physiological resistance decreases and although progesterone levels are in normal limits, uterus' exaggerated response leads to pyometra formation. Studies carried out in different regions indicated, pyometra is seen in Collie, Rottweiler, Cavalier King Charles Spaniel, Spitz, Doberman Pincher, Bernese Mountain Dog and Golden Retriever breed in the very high frequency [21,25,26] and pyometra develops with a higher frequency of growth rate in small breeds [26]. Unlike other studies in this study has made us think that the reason for Terriers (63%) being the dominant breed is connected to the cases refered to the clinics and dominant population of the dog breed in Turkey.

Escherichia coli isolated in both groups of cases to have high levels of toxins verify in the bacteria secreted major levels toxins. As in other studies, in this study AST and ALP values over reference ranges; ALT is close to the basal level in all dogs belong to Group I and Group II [19,27,28]. While AST, which is found in hepatocytes with important range; is releasing from mitochondria, ALT is releasing from cytocole. Therefore, serum ALT level is rising more easily than AST level. However, AST levels are also important in skeletal and heart muscle; it does not play a specific role in the liver destruction. In particular, bacterial endocarditis, aortic trombosis, infarction, cardiac and congesion and liver destruction are important factors cause increase in AST levels [29]. In this study, ALT and AST values were not parallel to increase supports the idea of the source AST origins from other tissues except liver tissue. Alkaline phosphatase is produced by liver, bone, intestine, kidneys and the placenta. Serum enzyme levels reflects, especially liver and bone tissue origined ALP. Unlike other enzymes growth in serum ALP levels is caused by the synthesis rather than cell destruction. Depending on the septicemia and endotoxemia following cholestasis, liver necrosis and inflammation, medium levels of ALP activity is observed [29,30]. As mentioned in other studies [15,21] ALP levels were thought to increase depending on kolestasis as a result of toxic hepatitis. Sevelius et al.[31] revealed that in 2/3 of dogs with pyometra had a destruction in renal paranchime and this situation was reflected in serum biochemical parameters. In the preset study, according to changes in BUN and creatinine levels, it was thought that at a rate of 33.3% in Group I and 66.6% of Group II had a destruction in renal paranchime. It has been reported that in 2/45 cases of dogs showed bad prognosis as a result of renal failure; increase in BUN and creatinine levels showed positive correlation (r=0.85) with blood ET level. In this study, the serum biochemical parameters of toxin level identified cases, generally, in cases with high toxin levels (0.96-1.92-3.84 EU/ml), BUN (62.7±44.3 mg/dL) and creatinine (2.5±2.2 mg/dL) levels

were found overall high above the reference intervals showed us the renal failure and the endotoxin levels were related to each other in pyometra as indicated in the literature [6,28,32]. As previously mentioned by other researchers [9,15,19] in all dogs belonging to both groups, a degenerative left shift with neutrophil finding was determined. The cases included in the study, the reason of anemia was thought to be due to the bone marrow supression by bacteria toxins and/or depending on the leap of erythrocytes from uterus resulting a decrease in erythropoiesis. As indicated in the literature [3,7,33], very high values of leucocyte and band neutrophils, supported the idea of development in inflammatory leucograms and absolute neutrophil with immature cells as a result of infection and septicemia in pyometra cases. In this study, the positive correlation rate between the survival rate and, toxin r=0.727 (P<0.01), BUN r=0.539 (P<0.01) and creatinine r=0.504 (P<0.05) levels attributed the importance of the relation between the increase in parameters and the prognosis of the disease. In dogs, it has been previously described that cardiac troponin I concentrations, indicating myocyte injury or myocardial damage were correlated to BUN, ALP, ALT and negatively correlated to lymphocytes. Also, a tendency for the association of detectable cardiac troponin I levels with high mortality rates was observed in the pyometra group [34]. In this study the phenomenon of death, with uremia was thought to be the result of a cardiovascular complication.

In recent years, surgical approach using an agent with antiendotoxemic drug to remove the endotoxin or neutralizing is the basic aim in pyometra treatment [35]. In this study, before and 7 days after operation, in anti-endotoxemic treatment group, the differences between the serum biochemical parameters were found trivial (P>0.05), in ampicillin applied dogs the differences betweenALT, ALP, BUN and creatinine levels were found important (P<0.05) suggested us the postoperative application of ampicillin was more effective on liver and renal functions. In the previous study, application of colistin-ampicillin in study group (n=15), ampicillin in control group (n=15) that was investigated the in effectiveness of polymyxin E in endotoxic shock developing dogs. In both groups of survived dogs, 2 hours after the treatment a decrease in the serum urea and creatinine levels and, from 2 hours in ampicillin applied group a gradually increase in this value, a continuous decrease in the polymyxin E-ampicillin applied group was seen respectively [36]. The difference of the findings obtained in this study from Şentürk's [36] regarded as the idea more study was needed. It is indicated that postoperative on day 2, the differences between average ALP, BUN, albumin and cholesterol levels was important (P<0.05) in dog with pyometra in comparison with the healthy dogs [15], other researchers established ALT and AST levels

reach in reference intervals in 2 weeks after the operation [28]. It has been reported that the increasing creatinine level was in reference interval postoperative on day 3 in dogs with pyometra [37]. It has been considered that solitary application of ampisillin had more efficacious effect on diminishing the suppative effect of toxins on bone marrow action than polymyxin E-ampicillin combination. This suggestion was based on the statistical significance in band neutrophile values in polymyxin E-ampicillin group and the significant differences in RBC, WBC, band neutrophile, Hct and lymphocyte values in ampisillin group. In addition, similar effects between application of polymixin E-ampisillin combination and ampisillin was observed in acute infections.

As a result, we concluded a relation between toxin levels and BUN-creatinine levels and a low survival in pyometra cases with increased toxin, BUN and creatinine levels; in contrast to the hypothesis, together with the operation ampicillin application was more effective than polymyxin E + ampicillin combination in bitches with suffering from pyometra.

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