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THE COMPARATIVE EVALUATION OF SERUM BIOCHEMICAL, HAEMATOLOGICAL, BACTERIOLOGICAL AND CLINICAL FINDINGS OF DEAD AND RECOVERED BITCHES WITH PYOMETRA IN THE POSTOPERATIVE PROCESS

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The aim of this study was to investigate the prognosis, the risk status and the death reasons in dead and recovered bitches with pyometra after the operative approach by comparing serum biochemical, and haematological parameters, as well as bacteriological and clinical findings. Bitches with pyometra (n=30) were ovariohysterectomized. Eight bitches that died after the ovariohysterectomy formed Group 1 and the rest, which recovered, was Group 2. In addition, 10 healthy bitches were used as the reference for haematological and biochemical parameters.

It was found that the level of band neutrophils in Group 1 was higher than 10% and there was lymphopenia along with a marked monocytosis resulting in a highly negative correlation (r=-0.74). There were significant differences (p<0.01) between Group 1 and 2 for BUN, creatinine and BUN/creatinine ratio. Also, the increased serum BUN and creatinine levels were significantly correlated ($r_{\rm s}=0.605$ and $r_{\rm s}=0.514$, respectively). A significant tendency was apparent for increasing mortality in bitches with pyometra whose BUN levels were over 30 mg/dL (odds ratio=23.80) and creatinine levels above 1.5 mg/dL (odds ratio=7.518). Clinical signs and bacteriological findings did not lead to marked differences between the groups.

In pyometra cases, the elderly azotemic dogs having higher BUN and creatinine concentration, would more likely succumb within 3 days after surgery. It may be concluded that the increased BUN concentrations may serve as a reliable prognostic predictor.

Key words: bacteriology, blood chemistry, canine pyometra, clinical signs, haematology, prognosis

INTRODUCTION

Pyometra is a common diestral disease in adult intact bitches characterised by genital and systemic illness with a variety of clinical and pathological findings

and inflammatory uterine accumulation (Johnston et al., 2001). In canine pyometra, the hormon-receptor relations, the virulence factors and toxins secreted by bacteriological agents and the importance of the binding capacity to the target organ receptors have been widely reported (Franson et al., 1997; De Bosschere et al., 2002). However, the central aspects of the complex etiology and the pathogenesis of the disease have not been completely explained yet. In bitches with endotoxemic and septic pyometra events, different organ systems and functions including homeostasis are impaired (Tanja et al., 2006). The sensitivity differences to endotoxic effects in species or individuals could be depended on the microcirculatory derangements, differential interactions of formed cellular elements with the activated vascular endothelium and normal physiologic reserves within organ systems (Pinsky and Rico, 2000). Many of the dogs with pyometra showed a cortisol pattern with neutrophilia, monocytosis and lymphopenia (Ohlson, 2005), Also, canine pyometra cases were accompanied with hypoalbuminemia, bilirubinemia, hypercholesterolemia and an increase in ALP and AST levels, and prerenal and renal azotemia (Noakes, 2001; Franson, 2003).

The clinical symptoms, bacteriological findings, haematology, blood biochemistry profiles and systemic inflammatory responses for the bitches with pyometra have been well described (Valoczky et al., 1998; Franson, 2003; Hagman, 2005). However, limited objective data is available on the prognostic significance of these profiles obtained before ovariohysterectomy and on the clinical outcome including the survival rates.

The purpose of this study was to determine whether preoperative hematological and biochemical parameters, and bacteriological findings and clinical symptoms have any indicative prognostic values on postoperative survival rate in bitches with pyometra; and to investigate the risk status and the possible death reasons after ovariohysterectomy in canine pyometra cases.

MATERIALS AND METHODS

The material consisted of 40 bitches from different breeds and ages, which were referred at the Obstetric and Gynecology Clinic, Faculty of Veterinary Medicine. The age of the animals ranged from 2 to 18 years and weights ranged from 5 to 32 kilograms. Diagnosis of pyometra was based on case history, clinical examination including ultrasonography and histopathologic evaluation of uterine tissues. Blood samples were collected for biochemical and haematological tests during clinical examination of all bitches (before fluid therapy and surgery). Samples for bacteriological examination were taken from the vagina and the vesica urinaria during surgery and from the uterus following ovariohysterectomy.

All bitches underwent ovariohysterectomy under standard general anesthesia protocols, rehabilitated in the hospital for five days after surgery. The surviving bitches were discharged on the 5th day of rehabilitation with instructions for the owners to give oral antibiotics according to antibiogram results for an additional 14 days. Surviving bitches were rechecked 21 days after surgery. For the replacement of fluid balance, intravenous fluid therapy was applied

presurgically and continued for 3 postoperative days combined with appropriate antibiotics.

The bitches were allocated into two groups. The animals (n=8) which died within 21 days after surgery composed Group 1 and the recovered animals (n=22) were described as Group 2. The third group consisted of 10 healthy intact animals and was used as the control for haematological and biochemical parameters.

Sampling and analyses

Blood samples were taken for testing biochemical and hematological parameters from the distal *cephalic vein*. Ethylendiaminetetra-acetic acide (EDTA) was used as the anticoagulant for biochemical analysis. Sera were separated and Asparate Amino Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP), Creatinine and Blood Urea Nitrogen (BUN) levels were determined. Hematologic parameters included erythrocytes (RBC), White Blood Cell count (WBC), haemotocrit (Hct), band neutrophils, segmented neutrophils, lymphocytes and monocytes. All the related parameters of surviving bitches were redetermined 21 days after surgery.

Swap and urine samples (via cystocentesis) were taken for bacteriological examination from the vagina and *vesica urinaria* during the operation and from the uterine lumen immediately after ovariohysterectomy, and then were cultured for aerobic bacterial isolation. Isolates were identified by using the conventional and Vitex automatic method (Biomerieux, France). The isolates' susceptibility to antimicrobial drugs was defined. The MICs were determined according to the standards of the National Committee of Clinical Laboratory Standards (NCCLS, 2000 and 2002), using Mueller Hinton broth (Oxoid, UK).

Statistical analysis

Statistical analyses was done using SPSS 14.01 program (serial: 9869264). One-way ANOVA (Duncan Posthoc) was used for comparison among the groups. Pearson's correlation (r_p) and Spearman's rank correlation (r_s) were used to test interrelationships in the data. Fisher's exact test was used for associations between BUN and creatinine levels and the mortality in pyometra groups. The level of significance for all statistical tests was p<0.01.

RESULTS

Bitches suffering from pyometra (n=30) were ovariohysterectomized and 8 of them died within 3 days after surgery (Group 1). However, because of owners' discontent, necropsy was not performed. The surviving 22 bitches (Group 2) were discharged from the hospital in healthy conditions. The results related to blood profiles, clinical and microbiological examination are presented in Tables 1 to 6.

Haematology and biochemistry

The findings for haematological and serum biochemical parameters are presented in Table 1. In Group 1 and Group 2, blood samples showed significant

haematological and biochemical differences than healthy animals in the control group. The levels of band neutrophils in all bitches in Group 1 were higher than 10%, compared with 8 bitches in Group 2. In Group 1, the correlation coefficient between the percentage of monocytes and lymphocytes was significantly negative $(r_p=-0.704)$.

Table 1. Haematological and blood biochemical parameters

	Group 1 Mean±SE	Group 2 Mean±SE	Control Mean±SE
n	8	22	10
RBC (10 ⁶ μl)	5.67±4.67 ^a	5.54±2.71 ^a	6.55±2.44 ^a
WBC (10 ³ μl)	42.40±7.24 ^a	35.40±5.62 ^a	8.39±6.74 ^b
Hct (%)	29.00±2.75 a	31.70±1.26 ^a	41.80±0.9 ^b
Band neutrophils (%)	18.25±2.48 ^a	18.54±2.56ª	2.80±0.5b
Segmented neutrophils (%)	64.75±2.96 ^a	65.77±2.65 ^a	68.50±1.04 ^a
Lymphocytes (%)	10.75±2.71 ^a	9.45±1.45 ^a	21.20±1.09 ^b
Monocytes (%)	8.75±3.32 ^a	6.40±0.88ª	4.20±0.59 ^b
AST (IU/L)	69.77±8.0 ^a	58.50±4.36 ^a	29.60±2.8b
ALT (U/L)	33.23±9.8a	24,92±2,8a	35.10±4.12 ^b
ALP (U/L)	205.12±41.16 ^a	211.13±43,3ª	83.50±4.62b
Creatinine (mg/dL)	2.71±0.82 ^a	1.15±0.13 ^b	0.96±0.09b
BUN (mg/dL)	70.83±15.68ª	27.76±5.05b	16.19±1.33 ^b
BUN/creatinine (mg/dL)	39.71±15.21ª	23.22±1.97 ^{ab}	18.26±0.91b

Means with different superscripts within a row are different (p<0.01)

BUN levels were determined to be above 25 mg/dL in all bitches in Group 1. There was a positive correlation ($\rm r_p{=}0.651$) between BUN and band neutrophils. In this group, creatinine levels in 5 of 8 bitches were above 1.5 mg/dL and had a negative correlation ($\rm r_p{=}{-}0.68$) with ALP. There were significant differences (p<0.01) for serum BUN, creatinine levels and BUN/creatinine ratio and there were positive correlations for BUN and creatinine ($\rm r_s{=}0.605$ and $\rm r_s{=}0.514$, respectively) between Group 1 and 2. In only 4 bitches in Group 2, both BUN and creatinine levels were above the reference values (Bush, 1996), while band neutrophil levels were below 10% in these bitches. The differences among the pyometra groups for AST and ALP values were not significant.

A significant trend toward increasing mortality in the pyometra bitches with 30 mg/dL or higher BUN level (odds ratio=23.80) and above 1.5 mg/dL creatinine levels (odds ratio=7.518) was observed.

The previous reproductive history, clinical signs and bacteriology

The means among the groups were similar, while the age distribution of bitches with pyometra was very wide, ranging from 2 to 18 years (Table 2). The animals were from different breeds, but the dominant breed was Terrier (63%). However, pyometra was not related to breed distribution (Table 2).

Table 2. Age and breed distribution in Group 1 and Group 2

	Group 1 (n= 8)		Group 2 (n=22)	
Age -Mean±SE Min-Max	10.63±0.804 7-18 years		10.14±1.19 2-15 years	
Breed	Terrier Doberman Pinscher Kangal (Local Turkish Breed) Cocker Spaniel Pekingese	4 1 1 1	Terrier Collie Rottweiler Poodle Labrador retriever Pekingese	15 3 1 1 1

The intervals between the onset of the last proestrus and the detection of pyometra signs ranged from 1 to 2 months in 5 of 22 animals in Group 1 and 4 months to 4 years in the remaining bitches. In Group 2, the same intervals were 1 to 3 months (14 animals) and 4 months to 7 years in others, respectively. In both groups, there was no evidence between the previous number of pregnancies, hormone treatments and the onset of the disease (Table 3).

Table 3. The details of parity, hormonal therapy and the onset of disease in the pyometra patients

		Group 1 (n=8)	Group 2 (n=22)
The interval between the onset of pro-estrus and detection of pyometra	1-3 month	5	14
		2	6
	>=1 year	1	2
Intervals between the detection of disease and the referal to the clinic, Mean ± SE (days)		17.28±4.99	35.60±12.74
Number of parturitions	Nulliparous	6	15
	Primiparous - Multipa rous	2	7
Hormonal therapy		1	1

All of the animals in pyometra groups showed signs of anorexia, polyuria / polydipsia. The differences in regard to clinical signs were not significant between both groups (Table 4).

Table 4. Clinical signs of pyometra bitches

		Group 1 (n=8)	Group 2 (n=22)
Anorexia		8	22
Polyuria - Polydipsia		8	22
Vomiting		2	10
Mild to severe depression		8	15
Vaginal discharge	Open cervix pyometra	6	14
	Closed cervix pyometra	2	8

The bacterial agents isolated from the uterus, vagina and *vesica urinaria* are presented in Table 5 and Table 6. A total of 90 samples were taken from animals with pyometra and 62 of them were found positive for pathogen agents. *E. coli* was isolated from the uterus of 4 cases, coagulase positive staphylococci from 2 cases and *P. aeruginosa* from only one case in Group 1 (Table 5). Similarly, in Group 2, *E. coli* was identified from uteri of 13 cases, β *haemolytic streptococci* from only one case, mixed infection from 2 cases, and other gram negative agents from 4 bitches (Table 6). The differences between the pyometra groups for pathogen agents were not significant.

Table 5. Bacteria isolated from uterus, vagina and *vesica urinaria* in the bitches which died after surgery

	Positive bacteriological growth / Total number of samples			
	Uterus	Vagina	Vesica urinaria	Total
No of Samples Positive growth	8 7	8 5	8 4	24 16
	Proportions of bacteria isolated from the uterus, vagina and v. urinaria (Number of the isolates /Total numbers of positive samples)			
Bacterial isolates	Uterus	Vagina	Vesica urinaria	Total
E. coli	4/7	3/5	2/4	9/16
Coagulase positive Staphylococcus	2/7	2/5	1/4	5/16
P. aeruginosa	1/7	_	1/4	2/16

The similarity between the bacterial strains isolated from the uterus and the vesica urinaria of the same animals was 57.14% and 71.4% among the uterus and vagina in Group 1. These levels in Group 2 was 55% and 75%, respectively. All of the similar strains obtained from the uterus and vesica urinaria or from the uterus

and *vagina* of the same animals showed the identical resistance and susceptibility to antimicrobials.

Table 6. Bacteria isolated from uterus, vagina and *vesica urinaria* in the bitches recovered after surgery

	The positive bacteriological growth / Total number of samples			
	Uterus	Vagina	Vesica urinaria	Total
No of samples Positive growth	22 20	22 15	22 11	66 46
	The proportions of bacteria isolated from uterus, vagina and vesica urinaria (Number of the isolates /Total numbers of positive samples)			
Bacterial isolates	Uterus	Vagina	Vesica urinaria	Total
E. coli	13/20	8/15	8/11	29/46
P. aeruginosa	1/20	2/15	1/11	4/46
K. pneumonia	1/20	1/15	1/11	3/46
K. oxytoca	1/20	_	1/11	2/46
B. Hemolitic streptococ	1/20	2/15	_	3/46
Enterococ	1/20	1/15	-	2/46
P. mirabilis	_	1/15	_	1/46
E. coli + ß. hemolitic Streptococci	1/20	-	_	1/46
E. coli + P. aeruginosa	1/20	_	_	1/46

DISCUSSION

Hematology and biochemistry

The parameters including increased WBC, band neutrophils, monocytes, AST, ALP, BUN, creatinine and decreased Hct, lymphocyte, ALT in the pyometra groups were consistent with previous studies (Franson, 2003). However, the reflections of bacterial infections or toxemia on haematological and biochemical profiles were more prominent in Group 1 than Group 2.

In Group 1, band neutrophils were above 10% in all bitches with Hct below 35% in 7/8 cases and also monocytosis together with lymphopenia in 6/8 cases (r_p =-0.704). This indicates usually normocytic, normochromic and nonregenerative anemia and represents chronic inflammation and toxic effects of bacterial agents on granulocytes. Neutrophilic leucocytosis can be seen with a variety of inflammatory lesions of the renal system. Depending on the severity and chronicity of the lesion, neutrophilia can be variably associated with a "left shift" or a peripheral leucocytosis (Robertson and Seguin, 2006). The marked neutrophilic

leucocytosis, increasing age and lack of invasive procedures are associated with high mortality rate in human patients (Chang and Wong, 1991) and in dogs (Lucroy and Madewell, 1999).

The moderate increase of AST and ALP values in pyometra groups were significantly different (p<0.01) than in the control group and ALT levels were close to the basal level. But, the differences between Group 1 and Group 2 for AST, ALP and ALT levels were not significant. AST occurs in a wide variety of tissues, but with high concentrations in skeletal muscles, cardiac muscle and in the liver. This increase in AST activity is attributed to some destruction in these organs. It is well known that bacterial endocarditis, aortic thrombosis, infarction, cardiac congestion and liver defects are important outcomes of increased AST levels (Bush, 1996). In the present study, it was found that the serum ALT values were not increased in a parallel manner to AST values. This may be explained as the increase in AST can be not related only to the liver, but also to some other tissues, as well. ALP is produced by the liver, bone, intestines, kidney and placenta. The increase in plasma ALP activity can be due to the isoenzymes derived from the liver and bone tissue (Bush, 1996).

In the present study, azotemia was observed in the animals who died after surgery. In Group 2, seven of eight bitches had BUN levels above 30 mg/dL and six bitches had both high BUN and creatinine levels (creatinine values over 1.5 mg/dL). In Group 2, BUN levels in 5 of 22 bitches were over 30 mg/dL and only 4 of these bitches had both BUN and creatinine high levels. Band neutrophyl ratio were below 10% in the animals that had both high BUN and creatinine levels. BUN and creatinine levels in Group 1 were significantly different from Group 2 (p<0.01) and BUN levels in Group 1 were also highly correlated with band neutrophyles. It is well known that the impairment of renal function is a frequent complication of pyometra along with other multiple organ dysfunctions (Heine et al., 2007) and coagulopathies (Tanja et al., 2006). Azotemia in canine pyometra cases is due to renal lesions with interstitial fibrosis and tubular atrophy which is caused by bacterial agents (Heine et al., 2007).

Kidney failure in humans is associated to secondary specific complications with consequent hypertension/hypotension, anemia, uremic pericarditis and cardiomyopathy caused by the uremic state (Schiffrin *et al.*, 2007). In dogs, it has been previously described that cardiac troponin I concentrations, indicating myocyte injury or myocardial damage were correlated to BUN, AP, ALAT and negatively correlated to lymphocytes. Also, a trend for the association of detectable cardiac troponin I levels with increased mortality was observed in the pyometra group (Hagman *et al.*, 2005). In the present study, 6 of the animals in Group 1 died in the second day and two bitches died in 3rd day after surgery. It was found that the elderly animals, which preoperatively had over 30 mg/dL BUN, along with higher than 10% band neutrophyles showed a marked tendency toward increasing case fatality. Although necropsies were not made we suggest that cardiovascular complications caused by the uremic state would be primarily responsible rather than other organ dysfunctions as the causes of mortality within 3 days after surgery. This suggestion was based on AST and ALT levels, the time

course of this effect, and the recent literature reports (Hagman *et al.*, 2005; Schiffrin *et al.*, 2007; Tatematsu *et al.*, 2007).

During sepsis, impairment of endothelial function is recognized as one of the initial mechanisms in renal disease (Chieko *et al.*, 1991). Endothelial dysfunction in turn may contribute to cardiovascular mortality in mild renal insufficiency. The presence of renal dysfunction associated with endothelial impairment leads to activation of renin-angiotensin system, oxidative stress, elevated plasma asymmetric dimethylarginine (ADMA), low/high grade inflammation with increased circulating cytokines, and dyslipidemia, which are all common pathophysiological mechanisms that play a role in renal failure and cardiovascular disease. Moreover, elevated plasma ADMA, produced by smooth muscle cells and cleared by the kidneys, has been used as a marker of cardiovascular diseases in early nephropathy in human patients (Schiffrin *et al.*, 2007). Recently, a similar cardiovascular effect has been shown in induced renal failure in dogs, too (Tatematsu *et al.*, 2007).

Clinical signs and bacteriology

The age bracket of pyometra cases extended from 2 to 18 years. Also, pyometra risk was higher in bitches which did not give birth than those with one or more parities. These findings were in agreement with many previous reports (Egenvall *et al.*, 2001; Noakes, 2001). The claim that some breeds are more prone to pyometra than others (Niskanen and Thrusfield, 1998) was not observed in our study due to an insufficient number of patients. The period between the last proestrus bleeding and the onset of pyometra signs was similar to some previous reports (Tsumagari *et al.*, 2005; Ishiguro *et al.*, 2007). However, this time period was markedly longer in 11 bitches in our study than some researchers' reports (Tsumagari *et al.*, 2005; Ishiguro *et al.*, 2007). It should also be noted that information about the time of proestrus bleeding was obtained from owners and it is questionable how reliable those informations were. On the other hand, there were no significant differences between pyometra groups for age, breed, onset of the disease, previous number of pregnancies and hormone treatments.

The microorganisms isolated from the uteri of dogs with pyometra were mainly *E coli*, and there was no differences between the groups. However, it may be worth to point out that coagulase *positive staphylococci* were isolated in two dogs in Group 1. Moreover, in Group 1, haematological and serum biochemical values of animals with *coagulase positive staphylococci* and *P. aeruginosa* isolated from their uterus, were in agreement with animals which *E. coli* isolated from their uterus. As it is known, *S aureus* releases harmful proteins and enzymes to the cells which cause tissue destruction and severe clinical symptoms (Vehmas-Ali and Sandholm, 1995). In a porcine sepsis model, it was reported that *Pseudomonas*, *S. aureus* and *E. coli* infusions induced acute respiratory failure in sepsis (Goldfarb *et al.*, 2005).

The same microorganisms were isolated from uterus and *vesica urinaria* of 4 dogs in Group 1 and 11 dogs in Group 2. The isolated microorganisms were *E. coli, coagulase positive staphylococcus, P. aeruginosa, K. pneumonia* and *K. oxytoca.* These agents gave similar results in antibiograms, which may indicate

similar resistance and susceptibility. The similarity between the bacterial strains isolated from the uterus and *vesica urinaria* of the same animals has been previously discussed (Hagman and Greko, 2005).

CONCLUSION

It may be concluded that the elderly azotemic dogs having above 30 mg/dl BUN concentration along with over 10% band neutrophils would likely end up with mortality from cardiovascular complications within 3 days after surgery. In these cases BUN concentrations together with band neutrophil level may serve as a good prognostic predictor. However, in further studies, it may be worthwhile to consider plasma asymmetric dimethylarginine concentrations (ADMA) in azotemic dogs with pyometra.

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UPOREDNA ANALIZA BIOHEMIJSKIH, HEMATOLOŠKIH, BAKTERIOLOŠKIH I KLINIČKIH NALAZA KOD UGINULIH I OPORAVLJENIH KUJA NAKON OPERATIVNOG LEČENJA PIOMETRE

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SADRŽAJ

Cilj ove studije je bio da se odrede prognoza, stepen rizika i razlozi uginuća kod kuja koje su operativno lečene od piometre. U tom cilju su analizirani biohemijski i hematološki nalazi, bakteriološki rezultati i kliničke opservacije. Prvu grupu je sačinjavalo osam kuja koje su uginule nakon operacije a drugu 22 jedinke kod kojih je operativni zahvat bio uspešan. Kao kontrola, pri tumačenju biohemijskih i hematoloških rezultata, služilo je 10 zdravih kuja.

Dokazano je da je u prvoj grupi postojala izraženija neutrofilija sa mladim oblicima ćelija (nesegmentirani leukociti) sa limfopenijom i monocitozom. Značajne razlike između prve i druge grupe su utvrđene i za nivo kreatnina, BUN i odnos BUN/kreatinin. Ovi parametri su bili u pozitivnoj korelaciji. Mortalitet je bio daleko veći kod jedinki koje su imale koncentraciju BUN veću od 30 mg/dl i kreatinin veći od 1,5 mg/dl. Klinički znaci i bakteriološki nalazi nisu bili u jasnoj korelaciji sa procentom uginuća.

Naši rezultati ukazuju da je kod starijih azotemičnih pacijenata prognoza relativno nepovoljna i da nivo BUN može biti jedan od prognostičkih parametara.