HAEMOSTASIS IMPAIRMENT IN BITCHES WITH PYOMETRA

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(Received 3. May 2006)

It is well known that pyometra syndrome with endotoxemia and/or sepsis in bitches may impair different organ systems and functions including haemostasis, which may considerably influence the outcome if overlooked or underestimated. The present clinical study was carried out on thirty-seven clinical patients admitted to the Clinic for Small Animal Medicine and Surgery at the Veterinary Faculty in Ljubljana, Slovenia. Preoperative diagnosis was based on clinical signs, haematology results and ultrasonographic examination. Ovariohysterectomy was performed as final treatment.

The study is aimed at the effects of pyometra syndrome on haemostatic functions as well as at the comparison between haemostatic functions and haematology and biochemistry results. Acute phase reaction with leucocytosis, neutrophilia, lymphopenia, hypoalbuminemia and hyperfibrinogenemia was identified preoperatively and up to 24 hours postoperatively. Changes in the haemostatic profile – prolonged activated partial thromboplastin time, shortened thrombin time, increased concentration of D-dimeres and thrombocytopenia – which were confirmed in the study, suggest the development of disseminated intravascular coagulation (DIC), most likely due to influence of E. coli endotoxin. The changes of haemostatic profile and concurrent acute phase reaction suggest the connection between inflammation and coagulopathy. Attention and early recognition of haemostatic function impairment should be addressed in pyometra syndrome patient in order to be able to timely substitute coagulation and anticoagulation factors with fresh blood, fresh or freshly frozen plasma or added heparin when appropriate.

Key words: DIC, endotoxemia, haemostasis, pyometra

INTRODUCTION

Pyometra is a disease most commonly observed during the luteal phase of the oestrus cycle. The primary pathologic process is reflected in the development of cystic endometrial hyperplasia (CEH) with accumulation of fluid in the uterine...
lumen (Johnson, 1998). Serum concentration of progesterone in these bitches is normal; there are anticipations that CEH is a consequence of the irregularity of endometrial receptors for oestrogen and/or progesterone (Schoon et al., 1992; Faldyna et al., 2001; Stone, 2003; Root Kustritz, 2005). Because of the hormonal stimulation and sensitization of the endometrium the uterine lumen can be colonized by facultative pathogenic bacteria (Schoon et al., 1992), which are a part of normal vaginal flora and invade the uterus ascendently (Fransson et al., 1997). The most frequently isolated bacteria is E. coli, less often isolates are Streptococcus spp., Staphylococcus spp., Pseudomonas aeruginosa and Proteus mirabilis (Fransson, 2003; Root Kustritz, 2005). E. coli and other Gram-negative bacteria have lipopolysaccharids (LPS) in their cell wall. These lipopolysaccharids are known endotoxins and they aggravate symptoms of bacterial sepsis, classical symptoms of pyometra and are often responsible for fatal outcome (Fransson et al., 1997). Endotoxin is released as a result of bacterial growth or destruction of bacterial cell (Fransson et al., 1997; Kruth, 1998). In healthy animals, small amounts of endotoxin from the intestinal bacterial flora are absorbed and transported to the reticuloendothelial system in the liver, where it is detoxicated and eliminated. If the amount of endotoxin exceeds the clearance capacity of the liver, systemic effects are seen: stimulation of the immune system, haemorrhagic diarrhoea and vomiting, haemodynamic changes and finally endotoxic shock. Endotoxin triggers the production of inflammatory mediators, such as cytokines (tumour necrosis factor (TNF), Interleukin-1 (IL-1), IL-6, IL-8), lipid mediators (thromboxane, prostaglandins, platelet-activating factor (PAF)) and oxygen free radicals (Fransson, 2003). TNF appears to have a significant role in coordinating the inflammatory response and activating the cytokine cascade. Its concentrations generally correlate with the severity of the illness and outcome (Blackwell and Christman, 1996). Pro-inflammatory cytokines impair anticoagulant mechanisms and up-regulate production of procoagulant factors (such as the platelet activating factor – PAF) thus creating an imbalance between procoagulant and anticoagulant mechanisms, in favour of the former (Aderka, 1991; Grignani and Maiolo, 2000). One of the factors of the coagulation cascade – thrombin, in turn, can promote inflammatory responses. This creates a cycle that logically progresses to vascular injury as occurs in septic shock (Esmon et al., 1999).

TNF also activates polymorphonuclears (PMNs) and increases their chemotaxis and adherence to endothelial surfaces, which further damages vascular endothelia and exposure of tissue factors (Jochum et al., 1981; Aderka, 1991; Taylor, 2001). Furthermore, experimental animal models of Gram-negative septic shock show that a monoclonal antibody against tissue factors attenuates coagulopathy and protects against death (Taylor et al., 1991).

Activation of the intrinsic path of coagulation is also vasculary dependent. Contact of blood with rough surfaces activates Hageman factor, which accelerates further activation of factors XI, XI, VIII, X, IX, V and F3 and cooperates with complement activation. It also directly and indirectly increases vascular permeability, activates fibrinolytic mechanisms and the extrinsic system (Dodds, 1989).
PAF alone has a very important role in the development of thrombocytopenia and neutropenia in animals with Gram-negative bacterial infection (Tsuchiya et al., 1999; Diehl et al., 2000). It is released from leucocytes, thrombocytes and endothelial cells and provokes activation and aggregation of thrombocytes, activation, aggregation and chemotaxis of neutrophils (Kruth, 1998; Tsuchiya et al., 1999). Platelet aggregation of septic patients is impaired – sepsis decreases haemostatic function of circulating platelets (Yaguchi et al., 2004).

It is known that conditions like anaemia, glomerulonephritis, tubular changes and intrahepatic cholestasys may develop subsequent to pyometra (Borresen and Skrede, 1980, Stone et al., 1988; Kruth, 1998; Heiene and Moe, 1999; Stone, 2003; Root Kustritz, 2005). In the present study, we focused on disorders of coagulation profile and/or occurrence of disseminated intravascular coagulation (DIC) in pyometra in bitches. Complete blood count (CBC) and serum biochemistry were used to get a better insight in the health status of bitches. Furthermore, serum was used to examine the effect of possible endotoxemia and septicemia on other organic systems and check if there is any possible connection with haemostatic disorders.

MATERIALS AND METHODS

We examined the haemostatic profiles of 37 client-owned bitches with pyometra admitted to the Clinic for Small Animal Medicine and Surgery at Veterinary Faculty in Ljubljana, Slovenia. Preoperative diagnosis was made based on clinical signs (purulent discharge from vagina, polyuria, polydipsia, anorexia, apathy, fever, vomiting), ultrasonographic examination and haematology results. Final diagnosis was made during the operative procedure. The type of pyometra was not defined. The average age of bitches with pyometra was 9.1 ± 3.2 years, which was in accordance with statements of other authors (Faldyna et al., 2001). No breed predisposition could be observed.

All bitches were treated preoperatively with amoxicillin – clavulanic acid (Synulox, Pfizer) 17.5 mg/kg and with gentamicin (Gentamicin, Krka) 5 mg/kg. Metadon (Heptanon, Pliva) 0.2 mg/kg as an analgesic was used for premedication. Induction of anaesthesia was accomplished with midazolam (Dormicum, Roche) 0.2 mg/kg and propofol (Diprivan, Zeneca) 3 - 4 mg/kg. Dogs were intubated and anaesthesia was maintained with isoflurance (Isoflouran, Rhone-Poulenc Chemicals); fentanyl (Fentanyl, Janssen) 0.5 - 1 µg/kg and ketamine (Ketanest, Parke-Davis) 0.5 - 1 mg/kg were used for additional analgesia during ovariohysterectomy. Fluid therapy with composed Na-lactate was used intraoperatively (10-20 mL/kg) and in maintaining dose postoperatively. Antibiotic therapy was continued until the day after the procedure and analgesia was maintained with tramadol (Tramal, Bayer) 5 mg/kg and carprofen (Rimadyl, Pfizer) 4 mg/kg.

Blood samples were collected for the determination of the haemostatic profile, complete blood count (CBC) and biochemical profile. The first blood analysis (phase 1) was performed when the patients were presented at the initial
examination, the second (phase 2) immediately after the operation, the third (phase 3) 24 hours postoperatively and the fourth (phase 4) on the tenth day postoperatively at the removal of stitches.

Citrated plasma (1 part citrate: 9 parts blood) samples from v. cephalica antebrachii were collected for one stage prothrombin time (OSPT or Quick), activated partial thromboplastin time (aPTT), thrombin time (TT), fibrinogen concentration (FBG) and D-dimer concentration. Samples were centrifuged within 30 minutes from collection at 4°C, 2000 g, separated and plasma was stored frozen at -70°C until analysis.

Quick, aPTT, TT and fibrinogen concentration were established with AMELUNG KC1A (SIGMA DIAGNOSTICS, Germany) analyzer. D-dimer concentration was established with latex test.

CBC was performed with automatic laser haematology analyzer Bayer Technicon H*1 (Bayer Technicon, Germany).

The biochemical profile consisted of the following parameters: urea, creatinine, sodium, potassium, chloride, calcium, anorganic phosphate, total protein, albumin, activity of alkaline phosphatase (AP) and alanine transaminase (ALT). These parameters, except sodium, potassium and chloride, were measured with biochemical analyzer Technicon RA-XT (Bayer-Technicon, Germany). Natrium, potassium and chloride were measured with Ilyte analyzer (IL – Instrumentation Laboratory, USA).

Results were statistically processed with "SPSS for Windows". Statistically relevant changes of CBC profile were ascertained with variance analysis (ANOVA) for repetition values. Statistical relevance was p<0.05. Average values, standard deviation and variation coefficient were calculated with "Excel" software.

RESULTS

Except one patient who died postoperatively all bitches recovered well and were completely healthy ten days after surgery.

In all four phases Quick was within reference values. Among different phases, there were no statistically significant changes (Table 1).

Table 1. Haemostatic profile of bitches with naturally occurring pyometra (Mean ± SD)

<table>
<thead>
<tr>
<th>Phase</th>
<th>QUICK</th>
<th>APTT</th>
<th>TT</th>
<th>FIBRINOGEN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(s)</td>
<td>(s)</td>
<td>(s)</td>
<td>(g/L)</td>
</tr>
<tr>
<td>Phase 1</td>
<td>6.34 ± 0.60</td>
<td>14.5 ± 3.4</td>
<td>8.47 ± 1.44*</td>
<td>4.48 ± 0.72*</td>
</tr>
<tr>
<td>Phase 2</td>
<td>6.54 ± 0.78</td>
<td>15.2 ± 5.7</td>
<td>8.3 ± 1.54*</td>
<td>4.36 ± 0.71*</td>
</tr>
<tr>
<td>Phase 3</td>
<td>6.4 ± 1.04</td>
<td>14.5 ± 4.4</td>
<td>8.09 ± 1.52*</td>
<td>4.49 ± 1.2*</td>
</tr>
<tr>
<td>Phase 4</td>
<td>6.2 ± 0.93</td>
<td>13.2 ± 3.4</td>
<td>9.3 ± 1.56</td>
<td>2.62 ± 0.8*</td>
</tr>
<tr>
<td>Reference values</td>
<td>6.3 ± 1.44</td>
<td>12.3 ± 2.1</td>
<td>11.3 ± 2.8</td>
<td>1.47 ± 0.75</td>
</tr>
<tr>
<td>(Jacobs et al., 1995)</td>
<td>4.86 – 7.74</td>
<td>10.2 – 14.4</td>
<td>8.5 – 14.1</td>
<td>0.95 – 2.22</td>
</tr>
</tbody>
</table>

*(P<0.05) in comparison with phase 4
Table 2. Blood cell count in bitches with naturally occurring pyometra (Mean ± SD)

<table>
<thead>
<tr>
<th>Phase</th>
<th>WBC *10^9/L</th>
<th>RBC *10^12/L</th>
<th>HGB g/L</th>
<th>HCT L/L</th>
<th>MCV Fl</th>
<th>MCH pg</th>
<th>MCHC g/L</th>
<th>PLT *10^9/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1</td>
<td>30.4±17.7*</td>
<td>6.3±0.92</td>
<td>147.8±37.5</td>
<td>0.43±0.07</td>
<td>68.7±4.9</td>
<td>24.4±1.5</td>
<td>354.9±15.6*</td>
<td>184.4±107.6*</td>
</tr>
<tr>
<td>Phase 2</td>
<td>20.6±13.4</td>
<td>4.83±0.84*</td>
<td>114.4±20.8*</td>
<td>0.34±0.063*</td>
<td>69.9±4.6</td>
<td>23.8±1.4</td>
<td>341.3±19.3</td>
<td>153.1±98.7*</td>
</tr>
<tr>
<td>Phase 3</td>
<td>47.5±23.2*</td>
<td>5.75±0.76*</td>
<td>138.1±18.9*</td>
<td>0.41±0.065*</td>
<td>70.9±5.0</td>
<td>24.1±1.5</td>
<td>339.6±19.7</td>
<td>183.4±119*</td>
</tr>
<tr>
<td>Phase 4</td>
<td>14.3±4.2</td>
<td>6.23±0.72</td>
<td>150.4±21.3</td>
<td>0.44±0.064</td>
<td>70.8±4.2</td>
<td>24.1±1.3</td>
<td>340.3±19.3</td>
<td>433.1±307.3</td>
</tr>
<tr>
<td>Ref.</td>
<td>6 – 18</td>
<td>5.1 – 8.5</td>
<td>115 – 180</td>
<td>0.35 – 0.55</td>
<td>62 – 76</td>
<td>20 – 25</td>
<td>320 – 360</td>
<td>200 – 500</td>
</tr>
</tbody>
</table>

*(P<0.05) in comparison with phase 4; Ref.: Reference values of haematological analyzer Bayer-Technicon H*1

Table 3. Differential white blood cell count in bitches with naturally occurring pyometra (Mean ± SD)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Neut %</th>
<th>Lymp %</th>
<th>Mono %</th>
<th>Eos %</th>
<th>Baso %</th>
<th>LUC %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1</td>
<td>78.7 ± 6.1*</td>
<td>10.6 ± 4.1*</td>
<td>6.5 ± 2.3*</td>
<td>0.84 ± 1.25*</td>
<td>0.11 ± 0.09</td>
<td>2.97 ± 1.44*</td>
</tr>
<tr>
<td>Phase 2</td>
<td>78.6 ± 6.7*</td>
<td>11.3 ± 6.1*</td>
<td>6.4 ± 1.8*</td>
<td>1.32 ± 1.19*</td>
<td>0.17 ± 0.24</td>
<td>2.13 ± 1.16*</td>
</tr>
<tr>
<td>Phase 3</td>
<td>86.7 ± 3.3*</td>
<td>7 ± 2.9*</td>
<td>3.8 ± 1.2*</td>
<td>0.93 ± 1.24*</td>
<td>0.091 ± 0.094</td>
<td>1.35 ± 0.64*</td>
</tr>
<tr>
<td>Phase 4</td>
<td>64.3 ± 11.3</td>
<td>20.7 ± 8.3</td>
<td>4.9 ± 1.7</td>
<td>8.88 ± 4.7</td>
<td>0.13 ± 0.1</td>
<td>1.2 ± 1.06</td>
</tr>
<tr>
<td>Ref.</td>
<td>60 – 80</td>
<td>12 – 35</td>
<td>0 – 9</td>
<td>0 – 10</td>
<td>0 – 2</td>
<td>0 – 2.5</td>
</tr>
</tbody>
</table>

*(P<0.05) in comparison with phase 4; Ref.: Reference values of haematological analyzer Bayer-Technicon H*1
Activated partial thromboplastin time (aPTT) was above reference values in the first three phases, but the difference was not statistically significant; however in the last phase aPTT values were normal (Table 1).

Thrombin time (TT) was below reference values in the first three phases and statistically significantly lower than in the last phase, when it was inside the reference values (Table 1).

Fibrinogen concentration (FBG) was at all times above upper reference value and was significantly higher in the first three phases of the experiment (Table 1).

Table 4. Serum biochemical profile parameters of bitches with pyometra (Mean ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Phase 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mmol/L)</td>
<td>6.0±3.9</td>
<td>5.5±3.3</td>
<td>4.7±2.5</td>
<td>6.1±2.7</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>113.7±43.3</td>
<td>98.6±25.8</td>
<td>104.2±32.6</td>
<td>105.1±30.5</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>145.8±7.9</td>
<td>147.6±5.8</td>
<td>151.8±5.5</td>
<td>149.6±3.2</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>3.8±0.57*</td>
<td>4.1±0.53*</td>
<td>3.8±0.52*</td>
<td>5.1±0.56</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>110.0±8.8</td>
<td>113.9±7.3</td>
<td>117.5±6.1</td>
<td>114.2±2.7</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.35±0.66</td>
<td>2.08±0.22*</td>
<td>2.06±0.20*</td>
<td>2.25±0.20</td>
</tr>
<tr>
<td>An. phosphate (mmol/L)</td>
<td>1.24±0.44</td>
<td>1.67±0.45*</td>
<td>1.23±0.41</td>
<td>1.34±0.28</td>
</tr>
<tr>
<td>AP (U/L)</td>
<td>147.1±132.3</td>
<td>110.5±89.0</td>
<td>130.4±83.8</td>
<td>103.5±82.9</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>22.6±130*</td>
<td>17.4±8.2*</td>
<td>26.6±27.1</td>
<td>41.6±222</td>
</tr>
<tr>
<td>Protein (g/L)</td>
<td>68.2±7.2</td>
<td>55.7±8.3*</td>
<td>59.0±7.6*</td>
<td>68.8±8.4</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>23.4±3.7*</td>
<td>19.1±4.0*</td>
<td>20.8±4.2*</td>
<td>29.1±5.4</td>
</tr>
</tbody>
</table>

*(P<0.05) in comparison with phase 4

As the method for the establishment of D-dimer concentration is semi quantitative, we expressed the obtained results as percent of bitches with D-dimer concentration above reference values (less than 250 ng/mL). D-dimer concentration was above the upper reference value in the majority of bitches (55%) in the first phase (above 250 and even more than 2000 ng/mL). In the second phase the percent of bitches with high D-dimer concentration was lower (49%). The day after the operation, we noticed a higher percentage of bitches with...
D-dimer concentration above reference values (68%). Ten days after the operation the D-dimer concentration was again lower: 48% of bitches had D-dimer concentration within reference values.

Results of haematology, differential white blood cell count and the serum biochemistry panel are shown in Tables 2, 3 and 4, respectively.

**DISCUSSION**

**Coagulation profile**

Pyometra can result in minor damage or necrosis of hepatocytes because of intrahepatic cholestasys and bile pigment retention (Stone, 2003). Hepatocellular damage results in variable deficiencies of haemostatic proteins as their synthesis is impaired and their half-lives are often shortened by increased consumption (Dufort and Matros, 2005). The quick test, which perceives differences in activity of factors of the extrinsic and common path (Monce et al., 1995), was within reference values at all times of our study, although coagulation factors are synthesized in the liver (Dodds, 1989). Therefore, we suppose that there was only minor liver damage, which did not interfere with coagulation factor production. Furthermore, we may also conclude that there was no major tissue damage that could set free tissue thromboplastin and activate the extrinsic system.

Prolonged, but not significantly different, aPTT in the first three phases shows a moderate defect of intrinsic coagulation path. This defect was most likely influenced by endotoxins (From et al., 1975; Dodds, 1989). Because of normal Quick values, which is the most sensitive test for detecting liver damage (Dodds, 1989); prolongation of aPTT due to liver damage can be excluded. We detected increased activity of alkaline phosphatase in the first and third phase, this being most likely due to cholestasis. aPTT prolongation occurs consecutively because of septicemia, endotoxemia, vomiting and anorexia (Borresen and Skrede, 1980; Kruth, 1998; Stone, 2003) and was temporarily concealed because of fluid therapy in the second phase (Table 4).

Extrinsic and intrinsic coagulation systems are activated simultaneously after the application of endotoxin, but significant consumption of coagulation factors occurs more slowly (Aasen et al., 1978). We could not detect the depletion of coagulation factors; the reason may be in the early stage of the disease, namely, haemostatic and inflammatory stress and damage of microvascular endothelia become evident when E.coli and endotoxin concentrations are low, that occurs earlier than we can detect DIC by low fibrinogen concentration or other detectors of DIC (Taylor, 2001).

TT (time needed for the formation of fibrin after thrombin addition) shows qualitative and quantitative changes of fibrinogen. During the first three phases, TT was below reference values, which can be the result of high fibrinogen concentration in the first three phases.

Fibrinogen concentration is an important parameter in the diagnostics of DIC. Hypofibrinogenemia is characteristic for acute DIC phase and hyperfibrinogenemia for chronic DIC (Bick, 1988); the latter is far more often, most
likely because fibrinogen is the protein of acute phase reaction and as such the marker of systemic inflammatory reaction (Bateman et al., 1999). That could be the case also in our study, where fibrinogen concentration was above reference values during the first three phases and normal in the fourth phase (10 days after ovariohysterectomy). The acute phase reaction is the response of the organism to infection, inflammation and tissue damage (Johnson, 1999). Borrenson noticed acute phase reaction with concurrent hyperfibrinogenemia, hypoalbuminemia and hyperglobulinemia in pyometra patients (Borresen and Skrede, 1980), which coincides with some of our results: leucocytosis, neutrophilia, hyperfibrinogenemia and hypoalbuminemia. Gunzel-Apel et al. offer other explanations for high fibrinogen concentrations. They reported a significantly higher fibrinogen concentration during dioestrus in pregnant or nonpregnant bitches accompanied by a high progesterone concentration (Gunzel-Apel et al., 1997), which is most likely a local reaction of the coagulation system on the changes in uterine endothelium and epithelium during placentation (Bunck et al., 2001). Bitches in our research were presented 1-2 months after oestrous, in the luteal phase, and the high fibrinogen concentration during the first three phases can be explained by this mechanism. Serum progesterone concentration decreases significantly 7 days after ovariectomy (Gobello et al., 2001), which correlates with lower fibrinogen concentration in the fourth phase of our study.

D-dimer concentration was above reference values during all four phases. The highest portion (68%) of bitches with high D-dimer concentration was found 24 hours after the operation, which coincides with high fibrinogen concentration. Since D-dimer test is sensitive and specific test for DIC (Nelson and Andreasen, 2003), we can assume that high D-dimer concentration shows the development of DIC syndrome in bitches with pyometra.

Thrombocytopenia, evident during the first three phases, could be the result of increased consumption (DIC, endometrial bleeding), decreased production of thrombocytes in the bone marrow (Dodds, 1989) or mediated by endotoxin through PAF production (Kruth, 1998; Tsuchiya et al., 1999).

Some ascertained coagulation profile changes in our study such as prolonged aPTT, shorter TT, thrombocytopenia and increased fibrinogen and D-dimer concentration are consistent with some reported DIC changes such as: increased FDP or D-dimers, prolonged or normal aPTT and QUICK, thrombocytopenia, decreased antithrombin III activity (Moncé et al., 1995; Bateman et al., 1999). Based on these results we may assume that bitches in our study developed some degree of DIC syndrome.

Disturbances of the haemostatic system can cause local and diffuse bleeding, that can endanger the favourable outcome of the operation. Hypotension, hypothermia, plasma expanders and some other drugs used during the operation can additionally impair the function of the haemostatic system, which is already debilitated because of thrombocytopenia, thrombocytopathy and DIC and can further increase the probability of bleeding (Lipowitz et al., 1996). We did not clinically detect haemostatic disturbances immediately and 24 hours after the operation, which corresponds to the results of the investigation of Millis et
They found only a minor influence of ovariohysterectomy on the haemostatic profile 24 hours after the operation in healthy bitches (Millis et al., 1992).

**Haematological changes**
Leucocyte changes reported in dogs with pyometra in our, as well as in other studies, indicate stimulation of bone marrow by inflammatory processes, stress response and/or acute phase reaction (Millis et al., 1992; Kociba, 2000; Root Kustritz, 2005).
Erythrocyte diaphedesis into the lumen of the uterus, toxic depression of erythropoiesis in the bone marrow (Borresen and Skrede, 1980; Johnson, 1998; Root Kustritz, 2005) or erythrocyte destruction consistent with DIC (Dodds, 1989) may cause anaemia. Although there was no deviation of hematocrit, hemoglobin or erythrocyte number in phase one of our study, they were significantly decreased in phase two. Since none of the bitches suffered considerable blood loss, we assume that these lower values were the result of blood dilution by fluid therapy (Millis et al., 1992).

**Serum biochemistry profile**
Plasma protein changes are the result of acute phase reaction with hyperfibrinogenemia, hypoalbuminemia and hypolipoproteinaemia; immunoglobulins and coagulation factors do not change significantly (Borresen and Skrede, 1980). In our case, hypoalbuminemia was detected in the first three phases, but total protein concentration was normal. Sepsis and endotoxemia cause increased vascular permeability and therefore can contribute to albumin loss (Fantoni et al., 1999). Decreased calcium concentration in the second and third phase coincides with low albumin concentration.
According to high fibrinogen concentration (acute phase protein), leucocytosis with accompanying neutrophilia and lymphopenia in the first three phases of our study, the connection between inflammation and coagulation can be assumed although sepsis was not proven microbiologically.
The presence of bacteria and their toxins in the uterus, as well as the immune system of the macroorganism can cause damage to various organs (Borresen and Skrede, 1980; Stone et al., 1988; Kruth, 1998; Heiene and Moe, 1999; Stone, 2003). Our research detected only minor liver damage, evident by serum biochemistry changes, we did not detect any changes of urea or creatinine concentration. Ultrasound or intraoperative examination did not detect any further organ damage.

**CONCLUSION**
According to the results of this study, we would like to recommend that preoperative assessment of critically ill bitches with pyometra should include not only the standard haematological and biochemical profile, but also the haemostatic profile. This should be composed of aPTT, concentration of fibrinogen and D-dimers (or FDP). The results can identify possible hazards and improve our treatment options. When needed we could substitute coagulation...
and anticoagulation factors with fresh blood, fresh or freshly frozen plasma or add heparin in suspected or proven DIC syndrome.

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REFERENCES
POREMEĆAJI HEMOSTAZE KOD KUJA SA PIOMETROM

PLAVEC TANJA, CELINŠEK BARBARA, DOLINAR KRISTINA, PEČAR J, NEMEC ALENKA i BUTINAR J

SADRŽAJ


Studija je imala cilj da se prouči učinci sindroma piometre na hemostatsku funkciju kao i da se usporedite rezultati hemostatske funkcije sa hematološkim i biohemijskim nalazima. Reakcija akutne faze sa leukocitozom, neutrofilijom, limfopenijom, hipoalbuminemijom i hiperfibrinogenemijom bila je ustanovljena preoperativno i do 24 sata pooperativno. Promene u hemostatskom profilu – pro- duženo aktivirano delomično tromboplastinsko vreme, skraćeno trombinsko vreme, povećana koncentracija D-dimera i trombocitopenija – koje su bile ustanovljene u studiji, sugeriraju na razvoj diseminirane intravaskularne koagulacije (DIC), najverovatnije zbog utecaja endotoksina E. coli. Promene u hemostatskom profilu sa pratećom reakcijom akutne faze govore za povezanost upale i koagulopatije. Svesnost i rana detekcija smetnji u hemostatskoj funkciji kod sindroma piometre omogućuju pravovremenu substituciju koagulacionih i antikoagulacionih faktora sa svežom krvlju, svežom ili smrznutom plazmom, kada je to potrebno.