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Measurement of C-reactive protein and Prostaglandin $F_{2\alpha}$ Metabolite Concentrations in Differentiation of Canine Pyometra and Cystic Endometrial Hyperplasia/ Mucometra

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Contents
Canine pyometra is a dioestrus period disease in which systemic inflammatory response syndrome (SIRS) is a common outcome due to the response of the body to the bacterial infection. The purpose of this study was i) to differentiate canine pyometra and cystic endometrial hyperplasia (CEH)/ mucometra by measuring serum C-reactive protein (CRP) and prostaglandin $F_{2\alpha}$ metabolite (PGFM) concentrations in blood and ii) to compare serum concentrations of CRP and PGFM in bitches with a pathological uterus (pyometra or CEH/ mucometra) to concentrations in bitches with a healthy uterus. Mean CRP concentrations were found significantly higher ($p < 0.001$) in dogs with pyometra compared to those with CEH/mucometra or healthy uterus. However, no statistical difference could be detected between the groups for mean PGFM concentrations. Mean white blood cell count (WBC), alkaline phosphatase (ALP) and total protein concentrations were found significantly higher ($p < 0.001$) in dogs with pyometra. *Escherichia coli* was the most frequently isolated microorganism from dogs with pyometra (64.3%). *Edwardsiella spp*. was detected in a single case of pyometra for the first time. In conclusion, our results demonstrate that serum CRP concentrations were increased in dogs with pyometra and thus we conclude that serum CRP concentration but not PGFM might be useful as a marker to differentiate a case of CEH/ mucometra from pyometra in female dogs. To the authors' knowledge, this is the first report in which *Edwardsiella spp*. has been isolated in the canine uterus.

Introduction

Canine pyometra (chronic purulent endometritis) is a dioestrus period disease in middle-aged and old bitches in which systemic inflammatory response syndrome is a common outcome due to the response of the body to the bacterial infection (Hardy and Osborne 1974; Purvis and Kirby 1994; Hardie 1995; Brady and Otto 2001; Egenvall et al. 2001). The clinical signs of the disease depend on whether the cervix is open or closed and may include the presence or absence of a purulent vaginal discharge, abdominal pain and systemic signs such as lethargy, depression, anorexia, polyuria, polydipsia and vomiting (Hardy and Osborne 1974). On the other hand, cystic endometrial hyperplasia (CEH) develops after repeated progestational effect at the luteal phase of the cycle (Dow 1958). Although CEH is thought to predispose the bitch to develop pyometra (Dow 1958; Sandholm et al. 1975), it has been reported that these two conditions can develop independently of each other (De Bosschere et al. 2001). Nevertheless, dogs with CEH/mucometra should be clinically monitored since they may be incapable of eliminating the bacteria resulting in the survival of the bacteria in the cystic fluid of the uterus and eventually development of pyometra (De Bosschere et al. 2001; Bassessar et al. 2013). CEH can cause mucometra leading to endometrial thickening with the accumulation of viscid, sterile fluid in the uterus but in pyometra this fluid is contaminated with bacteria (Hardy and Osborne 1974; Pretzer 2008).

In a case of a closed pyometra, a delay in surgical approach may result in potential uterine rupture, peritonitis and sepsis which may lead to an increased risk for the animal's life. Clinically, it would be helpful to predict the definitive diagnosis before the surgery (Hagman et al. 2006a). Despite their clear differences in pathological examinations, CEH and pyometra can be difficult to distinguish clinically since fluid accumulation is observed in both of the diseases which can be determined by diagnostic imaging (Fransson et al. 1997; De Bosschere et al. 2002). Even though immediate OHE can be performed, biomarkers helping to make a decision would be helpful (Fransson et al. 2004).

Uterine tissue synthesizes and releases prostaglandins, mainly prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) during inflammation (Heap and Poyser 1975). The presence of $PGF_{2\alpha}$ can be detected by measuring its circulating metabolite 15-keto–(13,14)-dihydro-$PGF_{2\alpha}$ (PGFM) (Granstrom and Kindahl 1982; Hagman et al. (2006a,b) Hagman et al. 2009). This metabolite increases in dogs with pyometra and decreases after ovariohysterectomy (OHE) (Hagman et al. 2009). In pyometra, the analysis of plasma PGFM concentrations is important for differentiation of CEH/mucometra from pyometra for evaluating the outcome not only in dogs but also in cats (Hagman et al. (2006a,b); Hagman et al. 2009).

Acute phase proteins (APPs) consist of a group of proteins including CRP, haptoglobin, ceruloplasmin whose concentrations increase (positive APPs) or decrease (negative APPs) due to inflammation (Ceron et al. 2005). An increase in CRP concentration may be an indication of an inflammatory process and is not influenced by age and gender, steroid therapy or administration of non-steroidal anti-inflammatory drugs or an opiod, butorphanol (Paul et al. 2011). In dogs, CRP concentration may increase due to various infectious, traumatic and neoplastic and immune mediated conditions resulting in an inflammation (Conner and Eckersall 1988; Onishi

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et al. 2000; Fransson et al. 2004; Holm et al. 2004; Ceron et al. 2005; Tecles et al. 2005; Mischke et al. 2006; Nakamura et al. 2008). Canine CRP was found to be free from circadian rhythm and day-to-day variations in healthy dogs (Otabe et al. 1998). Elevated concentrations of CRP have been previously reported in bitches with pyometra in blood samples prior to the surgery (Dabrowski et al. 2009) and thus proposed to be a useful marker in pyometra and/or other diseases leading to systemic inflammation (Jitpean et al. 2014). The purpose of this study was i) to differentiate pyometra and CEH/mucometra by measuring serum CRP and PGFM concentrations in blood and ii) to compare serum concentrations of CRP and PGFM in bitches with a pathological uterus (pyometra or CEH/mucometra) to concentrations in bitches with a healthy uterus.

Materials and Methods

Animals

A total of 35 privately owned bitches of various breeds and weights (3–43 kg) were used in this study. All procedures were approved by the Istanbul University Animal Researches and Ethic Committee (2012/19, 15.02.2012) and the consent of each owner was obtained. The dogs were divided into three groups: group A included 15 bitches suffering from pyometra (5–35 kg; median age was 9.3 years), group B: 10 bitches with CEH/mucometra (3–35 kg; median age was 9.7 years) and group C: 10 healthy dioestrus bitches (as control: 3–43 kg; median age was 3.7 years). All dogs were presented to Istanbul University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology clinic. A tentative diagnosis was based on signalment, case history, physical examination, vaginal cytology, complete blood count (red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), white blood cell count (WBC), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), serum biochemistry (glucose, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin) and transabdominal ultrasonography (Easote Piemedical MyLab Five Vet, Netherlands). The owners of the dogs, used as controls, were kindly asked to fill in a questionnaire confirming that the dog was clinically healthy and was not on any medication for at least 3 months prior to the presentation. Two dogs from group A and three dogs from group B were primiparous bitches. Only bitches in dioestrus determined by anamnesis (at least 1 month after the oestrous), vaginal cytology (presence of non-cornified vaginal cells and possible polymorphonuclear cells) and transabdominal ultrasonography (thickened endometrium and thicker walled structures on the ovaries) were used in the control group. Upon transabdominal ultrasonography, the appearance of the endometrium, presence of any free fluid and cystic hyperplasia of the endometrial glands were evaluated. CEH was ultrasonographically diagnosed when the endometrium was thickened, contained cystic structures in the uterine wall with cystic glands. When some amount of fluid was present within the uterine lumen, it was considered as mucometra. Presence of enlarged uterine diameter, a lumen filled with hypoechoic fluid and a thin uterine wall was classified as a case of pyometra. An ovariohysterectomy was performed on each dog on the same day of the presumptive diagnosis and the tissues were submitted to pathology for a definitive diagnosis. After the surgery, diagnosis was verified by gross examination for pus accumulation and the submitted tissue samples were evaluated by pathologists to confirm the diagnosis and thus to group the cases correctly. None of the control group dogs exhibited signs of pseudopregnancy after the surgeries.

Blood collection and analyses

Before the surgery, blood samples were collected for haematological, biochemical, CRP and PGFM analyses. Serum biochemical parameters included alanine amino transferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine, glucose, albumin, total protein and alkaline phosphatase (ALP) concentrations were all analysed in our faculty's laboratory according to routine methods before the surgery. Blood samples for measuring CRP and PGFM concentrations were centrifuged for 15 min at 1000 × ***g***, serum was transferred to microcentrifuge tubes and was stored at −20°C until assayed. Serum concentrations of CRP and PGFM were determined by quantitative sandwich enzyme immunoassay technique (μQuant, Bio-Tech Instruments, High Point, NC, USA) using commercial kits (CRP; DRG CRP canine ELISA, EIA-4694; DRG International, East Mountainside, NJ, USA; PGFM; Dog 15-keto-13,14-dihidro-prostoglandin $PGF_{2\alpha}$, CSB-EQ027222DO; Cusabio Biotech Co., Wuhan, China) according to the manufacturer's instructions. The detection range of CRP was 3-25 μL and the intra- and interassay coefficient of variations (CV) were 1.8–3.0%. The detection range of PGFM was 1.56–100 pg/ml and the intra- and interassay coefficient of variations were < 8% to < 10% respectively.

Bacteriological examination

Samples for bacteriological examination were obtained immediately after the surgery using sterile swabs from each uterine horns not only from the fluid accumulations but the swabs were additionally rubbed to the uterine horns' walls. The samples were inoculated into Nutrient broth (Oxoid Ltd., Cheshire, UK) with 5% horse serum added, on blood agar (Blood Agar Base, with 7% defibrinated sheep blood; Oxoid Ltd.) and MacConkey agar (Oxoid Ltd.) plates. Inoculations onto blood agar plates were duplicated and incubated both aerobic and micro-aerobic conditions. MacConkey agar plates were incubated aerobically; Nutrient broths were also incubated microaerobically at 37°C for 72 h. For anaerobic examination, the samples were cultured on Fastidious Anaerobe Agar (FAA, LabM, London, UK) plates, onto Nutrient Agar with 7% sheep blood, and incubated at 37°C for 5 days using jars and an

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anaerobic gas-generating kit (Oxoid Ltd.). Gram staining was performed from the cultures and bacteriological methods were used for the identification (Quinn et al. 1999). In this study, neither qualitative nor quantitative presence of the mycoplasmas and ureaplasmas was investigated.

Histopathological evaluations

Following OHE, surgically removed tissues were submitted for histopathological examination. Tissue samples were fixed in 10% buffered formalin, routinely processed, embedded in paraffin. From paraffine block 4 μm sections were cut and stained with haematoxylin and eosin (H&E) and examined under light microscope. Samples showing only hyperplasia and cystic changes in glandular structures with or without slight cellular infiltration between glands were classified as cystic endometrial hyperplasia; samples demonstrating hyperplasia, cystic changes in glandular structures and with moderate to high cellular infiltration between glands and lumens as well as the presence of pus within the uterine lumen were classified as pyometra; samples with hyperplastic and cystic endometrium with serous or mucoid fluid accumulation in the lumen were classified as mucometra and the samples showing thickened endometrium and oedematous stroma with secretions in the glands were classified as healthy bitches at dioestrus stage (Fig. 1).

Statistical analysis

The results were analysed with One-way ANOVA and Duncan tests between the groups. Pearson correlation analysis was performed between the biomarkers of complete blood count, serum biochemistry results and mean serum PGFM and CRP concentrations but not within the groups and a p-value of <0.05 was considered significant.

Results

Thirteen different breeds were represented, terriers were the most commonly affected breed with uterine pathologies (n = 8). The cervix was open and vulvar discharge was present in 11 of the 15 bitches in group A (73.3%) and 4 of the 10 cases (40%) in group B. The duration of the clinical symptoms and vulvar discharge prior to admission to hospital was 10 days (n = 2), 15 days (n = 8), 21 days (n = 1), 25 days (n = 1), 30 days (n = 2) and 45 days (n = 1) in the bitches from Group A. In group B, 4 dogs had vulvar discharge for 3 days prior to admission to hospital. The clinical data of the bitches used in this study are presented in Table 1.

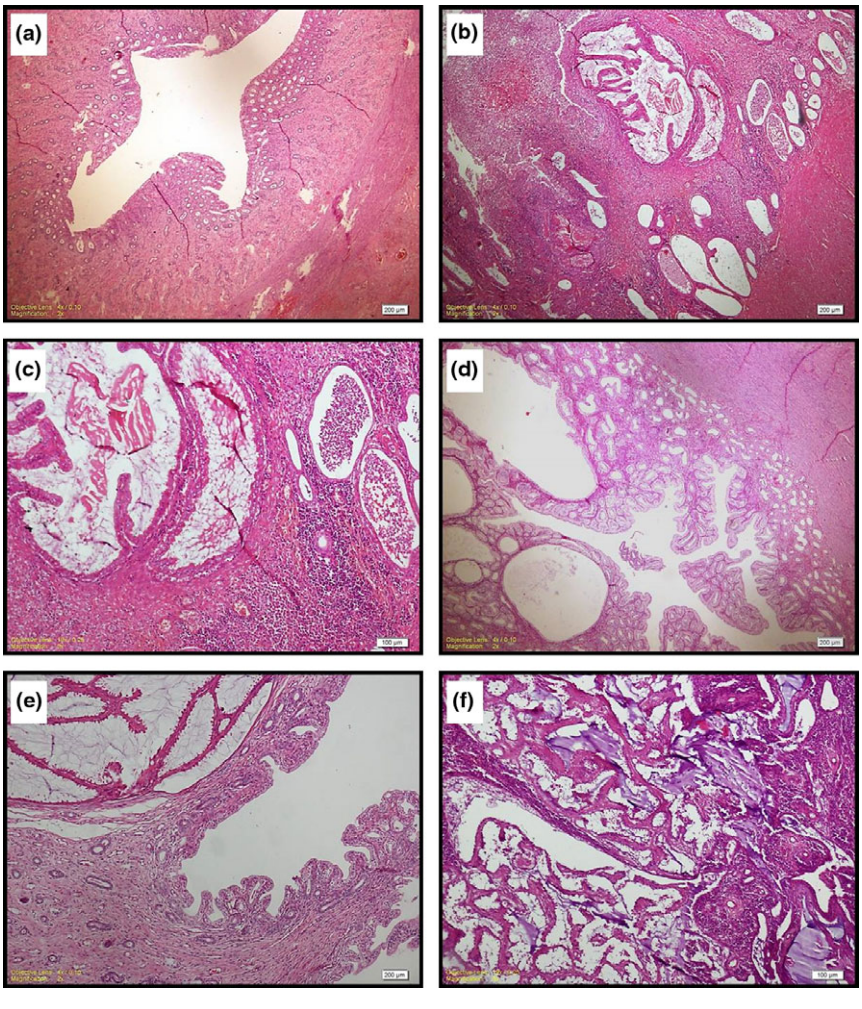

Fig. 1. (a) Dioestrous uterine tube at least one month after the oestrous; decreased number of mucosal folds, mildly proliferative endometrial glands. Hematoxylin–eosin. (b) Pyometra following cystic endometrial hyperplasia; luminal and inter glandular inflammatory cells, cystic changes in the glands and papillary changes in glandular epithelium. Hematoxylin–eosin. (c) Pyometra following cystic endometrial hyperplasia; luminal and inter glandular inflammatory cells and papillary changes in glandular epithelium. Hematoxylin–eosin. (d) Cystic endometrial hyperplasia, cystic glands, hypertrophy in endometrial glands, hyperplastic epithelium with papillary changes. Hematoxylin–eosin. (e) Cystic endometrial hyperplasia, cystic glands; hyperplastic glandular epithelium with papillary changes. Hematoxylin–eosin. (f) Mucometra; cystic and hypertrophic glands with blue mucoid content. Hematoxylin–eosin

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Table 1. Clinical data of 35 female dogs used in this study

	Breed	Age (year)	PD	PU	VD	L	A	Previous hormonal treatment*	Previous parturition
Group A									
1	Pekingese	9	√	X	√	√	√	√	√
2	German Shepherd Dog	11	√	√	√	√	√	X	X
3	Norfolk Terrier	15	X	X	√	√	√	√	X
4	German Shepherd Dog	9	√	√	√	√	√	X	X
5	Golden Retriever	8	√	√	√	√	√	X	X
6	Golden Retriever	5	√	√	√	√	√	√	√
7	English Cocker Spaniel	6	√	√	√	X	√	X	X
8	Golden Retriever	8	√	√	X	√	√	X	X
9	Pekingese	7	√	√	√	√	√	X	X
10	Border Terrier	7	√	√	X	√	√	X	X
11	English Cocker Spaniel	12	X	X	X	X	X	X	X
12	Border Terrier	13	√	√	√	√	√	X	X
13	Kangal dog mix	7	√	√	X	√	√	√	X
14	Golden Retriever	10	√	√	√	√	√	X	X
15	English Setter	13	√	√	√	√	√	X	X
Group B									
1	Border Terrier	15	X	X	X	X	X	X	√
2	Miniature Pincher	9	√	X	X	X	X	X	X
3	Golden Retriever	2	X	X	X	X	X	X	X
4	Golden Retriever	4	X	X	X	X	X	X	X
5	Border Terrier	12	X	X	X	X	X	X	√
6	Norfolk Terrier	8	X	X	√	X	X	X	X
7	Husky	7	X	X	√	X	X	X	X
8	Norfolk Terrier	17	√	√	√	X	X	X	X
9	Rottweiler	9	√	√	√	X	X	X	√
10	Albino Cairn Terrier	14	√	√	X	X	X	X	X
Group C									
1	Miniature Pincher	1.5	X	X	X	X	X	X	X
2	Beagle	2.5	X	X	X	X	X	X	X
3	English Bulldog	2	X	X	X	X	X	X	X
4	Golden Retriever	10	X	X	X	X	X	X	X
5	Beagle	2	X	X	X	X	X	X	X
6	Kangal Dog	2	X	X	X	X	X	X	X
7	Miniature Pincher	3	X	X	X	X	X	X	X
8	Cavalier King Charles Spaniel	1	X	X	X	X	X	X	X
9	Rottweiler	4	X	X	X	X	X	X	X
10	German Shepherd Dog	9	X	X	X	X	X	X	X

PD, polydipsia; PU, polyuria; VD, vulval discharge; L, lethargy; A, anorexia.
*Progesterone compounds to suppress oestrous signs.

Mean CRP concentrations were significantly higher ($p < 0.001$) in group A bitches than those determined in group B and C bitches. Although no statistical significance could be detected between the groups for mean PGFM concentrations, it seems that higher PGFM concentrations were obtained in pyometra cases compared with CEH/mucometra and control dogs (Table 2).

Serum CRP concentrations showed a negative correlation with serum PGFM concentrations. Furthermore, serum PGFM concentration showed a negative correlation with WBC count.

Mean RBC, HGB, HCT concentrations were significantly lower ($p < 0.05$) in group A when compared to group C. Mean WBC, ALP and total protein concentrations were significantly higher ($p < 0.001$) in group A than group B and C. Mean albumin concentration was significantly higher ($p < 0.001$) in group B and C than group A (Table 2).

Bacterial growth was observed in 12 of 15 samples collected from group A bitches. Single species of bacteria was isolated from 10 samples and two species from two samples. The most frequently isolated microorganism was *Escherichia coli* (64.3%), followed by *Klebsiella spp.* (14.3%), *Staphylococcus spp.* (14.3%) and *Edwardsiella spp.* (7.1%). Bacterial growth should not be expected in CEH cases; however, in three of 10 swab samples collected from group B bitches, bacterial growth was observed for one bacterial specie per swab which included *Staphylococcus spp.* and *Enterococcus spp., Staphylococcus spp.* followed by *Streptococcus canis,* although no inflammatory reactions were detected in the sections and CEH was confirmed histopathologically. Bacterial growth was not observed in any samples collected from the uterus of group C bitches (Table 3).

Histopathological examination of the surgically removed tissues revealed additional pathologies other than pyometra in four cases: follicular cysts on the ovaries and active corpus luteum, follicules on the ovaries and epithelial hyperplasia, chronic haemorraghic atrophic metritis and pus accumulation in the lumen and chronic necrotic metritis (each represented by one case). On the other hand, endometritis chronica cystica, severe haemorraghia on the mucosa layer, mild inflammation, mild plasma infiltration and only glandular

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Table 2. Mean CRP, PGFM and blood parameter results in Group A, B and C

Parameters	Group A (n = 15) Mean±SD error	Group B (n = 10) Mean±SD error	Group C (n = 10) Mean±SD error	Significance
CRP (μg/ml)	149 ± 25.38[a]	31.31 ± 11.43[b]	14.48 ± 4.93[b]	***
PGFM (pg/ml)	1.84 ± 0.82	1.51 ± 0.56	0.38 ± 0.03	NS
RBC (x10^6 μl)	5.36 ± 0.27[b]	6.38 ± 0.38[a]	6.58 ± 0.20[a]	*
HGB (g/dl)	11.78 ± 0.63[b]	14.71 ± 0.99[a]	15.25 ± 0.72[a]	*
HCT (%)	35.28 ± 2.03[b]	45.12 ± 2.66[a]	45.16 ± 1.96[a]	*
WBC (×10^3 μl)	44.38 ± 7.53[a]	16.18 ± 0.84[b]	13.92 ± 1.11[b]	***
PLT (×10^3 μl)	331.93 ± 55.07[b]	573.5 ± 112.42[a]	372.7 ± 55.93[ab]	NS
MCV	67.6 ± 1.00[b]	71.51 ± 1.01[a]	70.7 ± 1.11[a]	*
MCH (pg)	22.53 ± 0.36	23 ± 0.53	23.3 ± 055	NS
MCHC (%)	33.46 ± 0.33	32.3 ± 0.59	33.7 ± 0.49	NS
Glucose (mg/dl)	99.93 ± 6.55	107.9 ± 5.81	109.8 ± 5.62	NS
BUN (mg/dl)	59.86 ± 13.02	33.3 ± 6.08	31.7 ± 4.99	NS
Creatinine (mg/dl)	1.38 ± 0.24	0.88 ± 0.08	0.88 ± 0.07	NS
AST (IU/l)	55.86 ± 12.55	29.1 ± 3.08	42.4 ± 9.15	NS
ALT (IU/l)	28.33 ± 7.07	30.5 ± 1.91	40.3 ± 5.68	NS
ALP (IU/l)	387.06 ± 69.98[a]	55.7 ± 11.83[b]	102.9 ± 22.81[b]	***
Total Protein (g/dl)	7.60 ± 0.21[a]	6.25 ± 0.23[b]	6.58 ± 0.20[b]	***
Albumin (g/dl)	2.32 ± 0.13[b]	3.4 ± 0.33[a]	2.95 ± 0.13[a]	***

*p < 0.05, ***p < 0.001, NS: p > 0.05. a,b: Mean values within the same row with different superscript small letters are significant.

Table 3. The bacteria that have been isolated from the uterus of 35 female dogs

Isolated bacteria	Group A (n = 15)	Group B (n = 10)	Group C (n = 10)
E. coli	8	–	–
E. coli and *Klebsiella spp.*	1	–	–
Klebsiella spp. and *Staphylococcus spp.*	1	–	–
Staphylococcus spp.	1	1	–
Staphylococcus spp. and *Enterococcus spp.*	–	1	–
Streptococcus canis	–	1	–
Edwardsiella spp.	1	–	–
No growth	3	7	–

hyperplasia (each represented by one case) were also identified in dogs with CEH/mucometra.

Discussion

In this study, we aimed to differentiate cases of pyometra from those of cystic endometrial hyperplasia/mucometra by measuring the serum CRP and PGFM concentrations. Currently, there is no available literature including both PGFM and CRP analysis to differentiate the pyometra cases from those of CEH/mucometra, aiming to compare the efficacy of these two biomarkers to diagnose these diseases (CEH/mucometra or pyometra) as the underlying reason to add a new approach. Recently, Jitpean et al. (2014) investigated CRP, serum insulin-like growth factor-I, iron and serum amyloid A to predict the outcomes in dogs with pyometra and they concluded that CRP can be used as a prognostic marker for pyometra and/or other diseases leading to systemic inflammation. A lot of literature exists describing the use of kits to measure PGFM (Hagman et al. (2006a,b)) and CRP (Fransson et al. 2004; Dabrowski et al. 2007, 2009, 2013) with both ELISA and radioimmonuassay (RIA) methods in canine pyometra cases. In this study, we used quantitative sandwich enzyme immunoassay technique to measure the concentrations of PGFM and CRP in serum obtained from the bitches with uterine pathologies (CEH/mucometra or pyometra) and controls. Likewise, Fransson et al. (2004, 2007) used the commercially available canine sandwich ELISA kit in their study to measure CRP concentrations. Conversely, Hagman et al. (2006a,b) used radioimmonuassay (RIA) to analyse PGFM in dogs.

Mean serum CRP concentrations were found significantly higher in bitches with pyometra which was in accordance with those reported by Fransson et al. (2004, 2007). On the other hand, the mean serum CRP concentrations found in our study were higher than those reported by Dabrowski et al. (2007). This might be associated with the severity of the cases in this study since four bitches within the pyometra group had closed cervix pyometra whereas all cases reported by Dabrowski et al. (2007) had open-cervix pyometra. This discrimination suggests that accumulation of the purulent material within the uterine lumen may result in a more severe inflammatory response (Dabrowski et al. 2013).

In pyometra, bacterial infection and subsequent endotoxin release cause the endometrium to produce prostaglandins which is reflected by an increase in PGFM concentrations (Heap and Poyser 1975; Vandeplassche et al. 1991; Hagman 2004; Hagman et al. (2006a,b)). Hagman et al. (2006a,b) found significantly higher mean serum PGFM concentrations in dogs with pyometra (6278 pmol/l) compared to CEH/mucometra (415 pmol/l) and control (86 pmol/l) dogs. However, in this study, mean serum PGFM concentrations were found similar in dogs with pyometra (1.84 pg/ml), CEH/mucometra (1.51 pg/ml) or a non-pathological uterus (0.38 pg/ml). The difference between the studies might have several explanations. First of all,

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in their study *E. coli* was the primary bacteria isolated from 90% of the pyometra cases which might have resulted in significantly higher serum PGFM concentrations. In our study, *E. coli* could be detected only in 64% of the cases. Second, the method to measure PGFM concentrations in this study might have contributed to the insignificant difference between groups regarding PGFM measurements.

Laboratory tests, signalment, history and clinical findings are important when making a diagnosis. As expected, mean WBC concentrations were found significantly higher ($p < 0.001$) in dogs with pyometra when compared to control dogs and dogs with CEH. In this study, differential count of WBC including band neutrophils, eosinophils, basophils, lymphocytes and monocytes were not investigated according to the comments by Hagman et al. (2006a) about segmented neutrophils and monocytes as having low diagnostic value in differentiation of CEH/mucometra from pyometra. Conversely, Fransson et al. (2004) expressed the importance of the band neutrophils in combination with CRP concentration to predict the presence of pyometra. Mean concentrations of RBC, HGB, HCT were significantly lower ($p < 0.05$) in dogs with pyometra than the dogs with CEH/mucometra and control. Furthermore, in bitches with pyometra, serum biochemistry results including mean ALP, total protein concentrations showed a significant increase ($p < 0.001$). In accordance with our study, Fransson et al. (2004) reported similar results of complete blood count and serum biochemistry such as significantly ($p < 0.001$) decreased HGB concentrations, significantly higher ($p < 0.001$) WBC concentrations and significantly ($p < 0.001$) higher ALP concentrations in pyometra dogs when compared to healthy dogs and dogs with CEH. Fransson et al. (2004) found significantly lower albumin concentrations in bitches with pyometra than in bitches with healthy uterus. In this study, the same result was achieved. The reason of this result may be because of the decreased production and increased loss of this protein during systemic inflammation (Werner and Turnwald 1999).

In this study, *E. coli* (8/15) was the most commonly isolated bacteria from the uterus of bitches with pyometra which is in accordance with the previous literature (Bigliardi et al. 2004; Küplülü et al. 2009; Bondade et al. 2010; Bassessar et al. 2013). Other bacteria isolated in the current study were *Staphylococcus spp., Enterococcus spp., Streptococcus canis, Edwardsiella spp. and Klebsiella spp. Ewardsiella spp.* was detected for the first time in the canine uterus as well as in a case of pyometra in the current study. *Edwardsiella spp.* has been previously isolated from the intestines of healthy fish-eating birds, marine mammals, cattle, pigs and dogs (Songer and Post 2004). *Pseudomonas aeruginosa, Klebsiella pneumonia, Klebsiella oxytoca, β-hemolytic Streptococcus and Proteus mirabilis* are the other bacteria that have been isolated from bitches with pyometra (Küplülü et al. 2009). In a previous study (Bigliardi et al. 2004), no bacterial growth was observed in four uterine swab samples out of 45 cases with pyometra (9%) which is also seen in the current study (no bacterial growth in 3 pyometra cases of 15). Sterile bacterial cultures despite the presence of pus within the uterine lumen might occur due to the elimination of the bacteria by the uterine defence mechanisms of the host (Dhaliwal et al. 1998). Furthermore, the possibility of the influence of the method used in this study to collect samples for bacteriological examination on the negative bacterial growth in cases of pyometra cannot be excluded. In the current study, the bacteriological samples were collected by rubbing the swabs to the uterine horns' walls. Hagman et al. (2006b) aseptically removed 1×1 cm uterine wall sections for bacteriological examinations. Despite this difference in sampling techniques, bacterial growth was not detected in one case of 10 uterine samples in the study by Hagman et al. (2006b). In the study by Bigliardi et al. (2004), swab collection technique has not been mentioned in details. No bacteria were isolated in the samples obtained from 7 cases of 10 CEH/mucometra cases and any of the control cases. Although mycoplasmas were demonstrated as common inhabitants of the lower genital tracts of the female dogs and ureaplasma may have been recovered from the canine genital tract previously (Doig et al. 1981), mycoplasma and ureaplasma species were not identified in the current study because data are available indicating the presence of mycoplasmas over 60% of the dogs sampled clinically normal, besides ureaplasmas have been recovered from the canine genital tract but its possible pathogenicity is not clear (Doig et al. 1981). Kustritz (2006) have reported that mycoplasma and ureaplasma organisms are part of the normal flora of the canine genitourinary tract and in a study by Doig et al. (1981) no difference was detected in the rate of the isolation of mycoplasma and ureaplasma between clinically fertile bitches, infertile bitches, bitches with vaginitis and bitches that have no fertility history.

In conclusion, our results demonstrate that serum CRP concentrations increase in dogs with pyometra and thus measurement of this acute phase protein may be more reliable as a marker to differentiate between CEH/mucometra and pyometra in bitches rather than PGFM particularly in dogs with open-pyometra. However, it is upmost important that no other inflammatory disease should be present in the patient.

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Conflict of interest

None of the authors have any conflicts of interest to declare.

Author contributions

SO Enginler coordinated the project, collected the blood and tissues, and drafted the manuscript. A Ateş was responsible for to detect serum PGFM and CRP measurements. B Diren Sığırcı was responsible from microbiological examinations. BH Sontaş overworked on the manuscript. K Sönmez and A Gürel were responsible for pathological examinations. E Karaçam and H Ekici were responsible for surgeries and anaesthesia. G Evkuran Dal was responsible of drafting the manuscript.

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