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EFFECTS OF FEVERFEW (*TANACETUM PARTHENIUM*) AND ANTIBIOTIC CO-ADMINISTRATION ON BLOOD NEUTROPHIL FUNCTION AND SERUM PROCALCITONIN IN DOGS WITH EXPERIMENTAL SUBCUTANEOUS *PSEUDOMONAS AERUGINOSA* INFECTION

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ABSTRACT

The control of experimental canine *Pseudomonas aeruginosa* infection was optimised via a combined therapy including enrofloxacin against the bacteria and parthenolide (active component of the plant *Tanacetum parthenium*) for correction of inflammatory reaction. The aim of the present study was to evaluate the effect of this therapy on absolute neutrophil counts (ANC), blood neutrophilic function (phagocytosis, oxidative burst activity), serum naturally occurring antibodies (NAbs), procalcitonin (PCT) in infected animals. Blood samples were collected prior to the infection – hour 0 and post infection hours 4, 24, 48, 72 and day 7 from 25 male dogs, divided into five equal groups. Group C included healthy dogs (negative control). Dogs from Group 0 were injected subcutaneously with *P. aeruginosa* bacterial culture (1×10^8 CFU/mL) and untreated (positive control). The infected dogs from Group I were treated s.c. with enrofloxacin on post infection hour 48 at 5 mg/kg. Dogs from Group II were treated beginning from the 4th hour post infection with standardized feverfew extract – 90 mg, 0.7% parthenolide given orally at 2 capsules daily for 6 days. Group III included dogs with combined therapy - enrofloxacin and feverfew (at above mentioned doses and intervals). This therapy regulated ANC, NAbs, increased the phagocytic index, decreased hydrogen peroxide (H_2O_2) production of neutrophils and serum PCT. The observed correlations between PCT – marker of bacterial infection and blood markers – ANC, phagocytosis, H_2O_2 indicated that they could be used in combination to detect the progression of bacterial infection and can help to select the proper approach for treatment of infection.

Key words: Dogs, *Pseudomonas aeruginosa* infection, Neutrophil functions, Therapy.

INTRODUCTION

During the last years, the emergence of microbial pathogens resistant to numerous antimicrobial drugs is a serious problem. *Pseudomonas aeruginosa* is an element of the skin commensal flora of the dogs (Hoffmann *et al.,* 2014). This Gram-negative bacterium exhibits rapidly evolving antimicrobial resistance, biofilm formation (Breidenstein *et al.,* 2011). The choice of antimicrobial therapy in this infection is intricate (Kanj and Kanafani, 2011; Kmeid *et al.,* 2013).

The focus of therapeutic approaches used for treatment of *P. aeruginosa* infections are generally the antibiotics (Hillier *et al.,* 2014). The primary consideration in the selection of an antibiotic is clearly the sensitivity on the target microbial pathogen. Enrofloxacin, which is a third generation fluoroquinolone has good diffusion through bacterial membrane. After the entrance in the bacteria targets mechanism of its action has two major bacterial topoisomerase II (Gyrase) and the DNA topoisomerase IV (Topo IV) (Trouchon and Lefebvre, 2016). However, the potential of the antibiotic to interaction with the innate immune system of the infected host, may be of significance in some cases to affect the outcome of antibacterial therapy (Anderson *et al.,* 2010). Recent studies suggested that fluoroquinolones could enhance production of reactive oxygen species (ROS) in phagocytic cells, disturbed systemic oxidative balance and biofilm formation (Morita *et al.,* 2014). The most common side effect of enrofloxacin is an inflammatory reaction at the site of infection for injectable forms (Fauchier, 2013). *P. aeruginosa* also provokes a strong inflammatory response (Lavoie *et al.,* 2011). This bacterium inhibits the chemotaxis of neutrophils, suppresses the opsonins (both complement and immunoglobulin) and phagocytes, could rapidly react to abrupt changes in ROS (Lavoie *et al.,* 2011; Laarman *et al.,* 2012). The optimisation of strategies for control of *P. aeruginosa* infections requires combined therapy that includes an antibiotic against the pathogen and therapy that corrects the strong inflammatory response. Some researchers have used natural products with anti-inflammatory properties. The medicinal plant feverfew (*Tanacetum parthenium*), whose active principle is parthenolide, inhibits key signalling pathways of the inflammation – nuclear factor kappa beta (NF-kB),

prostaglandin synthesis, histamine, serotonin release (George *et al.*, 2012; Vitiello *et al.*, 2012). The phytopreparation also had antioxidant activity (George *et al.*, 2012). Although feverfew could be co-administered with antibiotics many important issues remain unresolved so this strategy will require demonstration of a favourable outcome from well-designed, multi-centre clinical trials.

The measurement of circulating, host-derived markers of inflammation and infection such as neutrophils and their functions, as well as procalcitonin (PCT) could be used to provide potentially valuable information regarding the pro-inflammatory status at the time of admission and during the course of antimicrobial therapy. The neutrophils, as mobile phagocytes, degrade internalised pathogens by phagocytosis (Kumar and Sharma, 2010). Phagocytosis is a relatively precise nonspecific antimicrobial effector mechanism that limits collateral damage to neighbouring host cells (Underhill and Ozynsky, 2002). It is dependent on several extracellular and intracellular factors – energy flow (Okpala *et al.*, 2015), expression of cell surface recognition receptors, opsonins (Donnelly and Barnes, 2012). Aung *et al.* (2015) provide a new perspective on the role of naturally occurring antibodies (NAbs) in the recognition of pathogens and the triggering of innate immune defense against bacteria. Hatzistilianou (2010) consider PCT as an early and rapid marker for detecting bacterial infection. Its half-life is 25 – 30 hours which makes it suitable for monitoring of the infection (Whicher *et al.*, 2001). The mechanism proposed for PCT production after inflammation and its role are still not completely understood.

The aim of the study was to evaluate the effect of combined therapy (parthenolide and enrofloxacin) on blood neutrophil counts, neutrophil function (phagocytosis and oxidative burst activity) and levels of serum NAbs and PCT concentration in dogs with experimental *P. aeruginosa* infection. A correlation analysis between studied parameters was made. We hypothesized that the *in vivo* inhibition of acute inflammation by parthenolide would alter the functional activity of neutrophils.

MATERIALS AND METHODS

Animals: This study was approved by the Ethical Committee at the National Veterinary Service (permit No. 37/2010) and was performed in accordance with animal welfare standards. A total of twenty-five healthy male, mongrel dogs, 2 – 5 years old, and average body weight 19.5±5.2 kg. were used. The dogs were from a kennel, healthy animals taking no medications were recruited based on the results of clinical investigations, laboratory parameters and parasitological investigations. The animals were individually housed in metal cages at the vivarium of the Department of General and Clinical Pathology, Faculty of Veterinary Medicine, under conditions of controlled air temperature (15-21 ^{o}C), air humidity – 50-60%, mixed lighting regimen. The animals were fed commercial canine dry food Canil Social Gouomarc H (Brasil), had free access to drinking water and were walked twice daily on a leash. The dogs were treated with Prazimec-D (Biovet Ltd, Peshtera, Bulgaria) against helmints at a dose of 1 tablet/10 kg. b.w. orally and Tapilan-B (Dorvert, Israel).

Experimental design: After adaptation period continued one month the animals were divided into five equal groups. Dogs (n=5) included in Group C (negative controls) were healthy and uninfected. Those from Group 0 (positive controls, n=5) were injected subcutaneously with *P. aeruginosa* bacterial culture (1×10^8 CFU/mL) into a depilated area of the neck region. Dogs from Group I (n=5) were infected and treated beginning from the 48th hour post infection with enrofloxacin (Syvaquinol 25 injectable, 2.5% solution – Syva laboratorios, Spain) given subcutaneously at a dosage of 5 mg/kg.b.w. Dogs from Group II (n=5) were treated beginning from the 4th hour post infection with 1 capsule feverfew every 12 hours daily (Feverfew standardized extract 90 mg, 0.7% parthenolide – Nature's Way, USA) given orally. The per os treatment began on post infection hour 4 and continued for 6 days. Dogs from Group III (n=5) were treated with a combination of enrofloxacin (5 mg/kg. b.w., subcutaneously) on post infection hour 48 and daily oral intake of two capsules feverfew beginning on post infection hour 4 for 6 days.

Bacterial strain: A field *P. aeruginosa* isolate from a dog, typed by the BBL Crystal semi-automated identification system (Becton Dickinson, USA) and kit for Gram-negative bacteria and non-fermentative bacteria was used. The presumptive identification of the strain was made on the basis of the following characteristics of *P. aeruginosa*: growth on MacConkey agar; pyoverdin production; oxidase test; growth at 5^{o}C and at 42^{o}C; oxidation of glucose, lactose, maltose; arginine dehydrolase production; reduction of nitrate to nitrite; urease production; gelatin hydrolysis.

Collection of blood samples: Blood samples were collected from *v. cephalica antebrachii* via Venflon cannulae (Vygon GmbH & Co., Germany - 20G) prior to the infection – hour 0 and on post infection hours 4, 24, 48, 72 and day 7. All blood samples were collected in the morning before feeding (8.00–8.30 AM) to eliminate circadian rhythms in heparinised tubes and serum separator tubes.

Blood and serum analysis: Total white blood cell (WBC) counts were obtained on an automated cell count analyzer (Coulter Electronics, Krefeld, Germany). Differential WBC counts were performed on stained smears. The absolute neutrophil counts were calculated

from WBC counts and the percentage of neutrophils obtained from the differential counts.

Percentage of phagocyting neuthrophils (PP) in whole blood samples – defined by the immunofluorescent method of Samnaliev *et al*. (1995). On the smear 150 neutrophils are counted. Then the parameter is defined by the formula:

% phagocyting cells = (count of phagocyting cells/150)x100

Phagocytic index (PI) – shows the mean number of engulfed bacteria by a phagocyting cell. PI = total count of engulfed bacteria/150. The immunofluorescence method of Samnaliev *et al*. (1995) has been applied as previously described (Andonova *et al.*, 2001).

The neutrophil oxidative burst was evaluated by the nitroblue tetrazolium chloride test (NBT) (Park, 1971), indicating the formation of hydrogen peroxide (H_2O_2) - a product of oxidative killing mechanism. H_2O_2 producing neutrophils was calculated as percentage of total neutrophil count (100) on a blood smear (safranin staining).

Serum naturally occurring antibodies were determined by nephelometric method (McEwan *et al.*, 1970).

Serum concentrations of canine PCT (pg/mL) were determined by ELISA kit (CA1033, TSZ ELISA, Scientific LLC, BIOTANG, USA), specially developed for dogs according to the manufacturer's instructions. Assay range was 30-1000 pg/mL.

Statistical analysis: Results are presented as mean±SD. Data were submitted to repeated measures ANOVA with post-hoc test (Graph Pad InStat 3). Differences were considered statistically significant at the $p<0.05$ level. The relationships among the different variables were examined by Spearman rank correlation analysis (MedCalc 10.2.0.0, Belgium).

RESULTS

The analysis of the data for absolute band neutrophil counts (ABC) in dogs (Table 1) showed that in animals from Group 0, the infection was characterised with statistically significantly increased ABC on the 48 hour ($p<0.05$ vs 0 h), 72nd hour ($p<0.001$ vs 0 h; $p<0.05$ vs Group C) and day 7 ($p<0.05$ vs Group C). In infected dogs treated with antibiotic (Group I), the increase was obvious on the 72nd hour ($p<0.05$ vs 0 h). A similar alteration has also occurred but earlier (on the 24th h – $p<0.01$ vs 0 h; vs Group C, and 48th h – $p<0.01$ vs 0 h; $p<0.05$ vs Group C) in dogs receiving only parthenolide (Group II). The peak attained by the 24th hour in this group was the highest among all groups. The combination of parthenolide and antibiotic applied to Group III kept ABC under control. The registered statistically significant deviations in absolute segmented (mature) neutrophil counts (ASC) consisted in increased counts by the 72nd hour ($p<0.001$ vs 0 h; $p<0.05$ vs Group C) in dogs from group 0, whereas in dogs from Groups I and II this parameter was elevated as early as the 24th post infection hour ($p<0.001$ vs 0 h) and persisted until the 72nd hour for Group I ($p<0.001$ vs 0 h) and for Group II ($p<0.05$ vs 0 h). In dogs from Group III, ASC were within the reference range (3-11.5 $x10^9$/L) throughout the period of the study.

The data from Table 2 showed that the percentage of phagocytosis (PP) decreased as early as the 24th hour in dogs from Group 0 ($p<0.001$ vs 0 h; $p<0.05$ vs Group C), Group I ($p<0.05$ vs 0 h) and II ($p<0.01$ vs 0 h; vs Group C). In all groups phagocytic activity of neutrophils remained lower vs Group C during the entire period of study. The possibility for clearing a higher number of opsonized bacteria by phagocytes was markedly outlined at the background of reduced phagocytosis. The phagocytic index (PI) was statistically significantly higher on hour 72 in Group I ($p<0.001$), II ($p<0.05$), III ($p<0.001$) as compared to dogs from Group C. The amount of naturally occurring antibodies (NAbs) (Table 2) changed statistically significantly only in Group 0, demonstrating increase on hour 48 ($p<0.01$ vs 0 h); hour 72 ($p<0.05$ vs 0 h) and at the study end – 7th day ($p<0.01$ vs 0 h). Unlike that, serum immunoglobulins were not considerably changed in infected dogs treated with enrofloxacin (Group I); phytopreparation feverfew (Group II) or both (Group III).

Hydrogen peroxide detected by NBT in dogs from Group 0 was increased statistically significantly by hour 48 ($p<0.05$ vs Group C; vs 0 h), 72 ($p<0.001$ vs Group C; vs 0 h), day 7 ($p<0.001$ vs Group C; vs 0 h) (Fig 1). The dogs from Group I showed considerably higher percentage of NBT by hour 48 ($p<0.01$ vs Group C; $p<0.001$ vs 0 h), 72 ($p<0.01$ vs Group C; $p<0.05$ vs 0 h). The application of parthenolide (Group II) or parthenolide with antibiotic (Group III), resulted in higher percentage of NBT-positive neutrophils compared to Group C, but lower percentages vs Groups 0 and I.

Serum PCT concentrations were increased in Group 0 ($p<0.001$ vs Group C), Group I ($p<0.01$ vs Group C), as early as the 24th hour (Fig. 2). In dogs from Group 0, the higher values persisted during the subsequent study intervals – hour 48 ($p<0.001$), hour 72 ($p<0.001$) and attained a peak on day 7. Unlike that, in dogs receiving enrofloxacin from the 48th hour onward (Group I), serum PCT concentrations declined gradually after the peak on the 24th hour. In dogs from Groups II and III, the elevation observed by the 24th hour persisted another 24 h later ($p<0.01$ vs Group C). Afterwards, the dogs from Group III responded with lower PCT levels unlike dogs from Group II, whose serum PCT concentrations increased - on day 7.

Correlation data are summarised in Table 3. The relationships among the different variables examined by

Spearman rank correlation analysis revealed a significant correlation of PCT – marker for bacterial infection with blood markers – ABC ($p<0.05$), NAbs ($p<0.01$) and H_2O_2 – detected by NBT, but exhibited an inverse relationship with PP ($p<0.05$). Among the various parameters of the blood neutrophil function, NBT showed a strong correlation with ABC ($p<0.01$), ASC ($p<0.01$) and NAbs ($p<0.001$). The phagocytosis (PP) showed a strong inverse correlation with ABC ($p<0.05$) and ASC ($p<0.05$). ABC showed a highly significant correlation with ASC ($p<0.001$).

Table 1. Changes in absolute band (ABC) and segmented neutrophil counts (ASC) ($x10^9$/L) in healthy dogs (Group C), dogs infected with *Pseudomonas aeruginosa* (Group 0) and infected dogs treated with: enrofloxacin (Group I); feverfew (Group II) and feverfew+enrofloxacin (Group III). Values are means ± SD of five dogs in each group

	Time post infection					
	0 h	**4 h**	**24 h**	**48 h**	**72 h**	**7 d**
Absolute band neutrophil counts (ABC) $x10^9$/L						
Group C	0.00±0.00	0.00±0.00	0.00±0.37	0.02±0.06	0.02±0.06	0.12±0.16
Group 0	0.10±0.10	0.45±0.52	0.39±0.42	1.51±0.86*	2.04±1.14 $^{***\ c1}$	0.82±0.44^{c1}
Group I	0.00±0.00	0.44±0.24	1.41±0.80	1.29±1.06	1.76±1.50*	0.64±0.16
Group II	0.09±0.16	0.58±0.37	2.10±1.10$^{**\ c2}$	1.80±1.16$^{**\ c1}$	1.53±0.96*	0.35±0.24
Group III	0.03±0.06	0.68±0.73	0.81±1.08	0.50±0.40	0.58±0.57	0.24±0.42
Absolute segmented neutrophil counts(ASC) $x10^9$/L						
Group C	5.12±1.36	5.52±1.12	5.72±1.48	4.74±1.88	5.63±2.18	6.00±2.80
Group 0	6.06±1.48	8.72±1.69	10.56±1.96	10.92±3.02^{c1}	16.34±4.26$^{***\ c1}$	9.56±2.62
Group I	3.10±0.94	8.66±1.70	13.26±4.06$^{***\ c1}$	12.90±4.15$^{***\ c2}$	16.82±6.27$^{***\ c2}$	9.98±1.60+*
Group II	5.54±2.20	9.76±2.62	17.71±5.92$^{***\ c3}$	13.94±3.38* c3	13.83±5.34*	6.64±2.20
Group III	4.98±2.90	9.52±4.62	11.02±4.20	11.37±2.10	9.58±3.56	6.72±5.06

Within-group statistical significance: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ vs 0 h; between-group statistical significance: 1 $P<0.05$; 2 $P<0.01$; 3 $P<0.001$; c – vs controls (Group C)

Table 2. Changes in percent of neutrophil phagocytosis (PP), phagocytic index (PI) and serum naturally occurring antibodies (NAbs) in healthy dogs (Group C), dogs infected with *Pseudomonas aeruginosa* (Group 0) and infected dogs treated with: enrofloxacin (Group I); feverfew (Group II) and feverfew+enrofloxacin (Group III). Values are means ± SD of five dogs in each group

	Time post infection					
	0 h	**4 h**	**24 h**	**48 h**	**72 h**	**7 d**
Percent of phagocytosis (PP) (%)						
Group C	30.0±2.2	28.8±5.5	29.2±6.0	29.0±7.4	30.2±2.6	28.8±6.5
Group 0	29.6±1.6	28.0±3.6	21.2±3.4$^{***\ c1}$	22.6±3.4**	25.0±2.2	27.0±2.0
Group I	29.0±2.9	24.2±3.1	22.2±4.4*	23.2±4.2	28.8±3.3	22.8±1.3*
Group II	28.4±4.6	23.8±4.2	19.2±1.3$^{**\ c2}$	26.4±3.2	22.2±1.4^{c3}	22.6±3.8
Group III	29.6±2.4	21.6±4.6	21.6±3.2	24.2±9.5	26.6±2.7	22.2±4.8
Phagocytic index (PI)						
Group C	0.82±0.12	0.78±0.23	0.82±0.22	0.80±0.20	0.71±0.05	0.74±0.30
Group 0	0.96±0.17	0.88±0.87	0.75±0.09	0.80±0.09	0.82±0.04	1.15±0.18^{c1}
Group I	0.88±0.10	0.89±.08	0.75±0.14	0.84±0.11	0.97±0.08^{c3}	0.80±0.06
Group II	0.91±0.13	0.89±0.16	0.72±0.03	0.98±0.10	0.84±0.06^{c1}	0.87±0.12
Group III	0.96±0.07	0.82±0.12	0.86±0.10	0.97±0.36	0.96±0.06^{c3}	0.82±0.13
Naturally occurring antibodies (NAbs) mg/mL						
Group C	25.19±3.79	26.22±2.63	23.70±2.40	24.60±1.06	25.02±5.86	26.10±3.15
Group 0	25.90±2.87	31.53±6.22	29.42±6.04	35.98±5.48**	35.20±4.17*	37.34±3.22**
Group I	27.24±2.93	22.46±5.45	25.79±9.64	28.06±12.6	33.90±15.7	35.41±6.22
Group II	27.02±3.20	33.67±9.70	26.08±10.8	30.43±13.2	31.21±7.23	31.57±11.7
Group III	26.51±3.04	26.20±7.70	25.71±9.15	25.22±5.95	29.18±5.65	30.70±4.61

Within-group statistical significance: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ vs 0 h; between-group statistical significance: 1 $P<0.05$; 2 $P<0.01$; 3 $P<0.001$; c – vs controls (Group C)

Table 3. Results of correlation analysis (correlation coefficients and levels of significance) between studied parameters in dogs.

	Parameters						
	ABC	ASC	PP	PI	NAbs	NBT	PCT
ABC	–	0.728***	-0.416*	0.255	0.443*	0.639**	0.506*
ASC		–	-0.514*	0.041	0.284	0.547**	0.309
PP			–	0.134	-0.157	-0.271	-0.489*
PI				–	0.288	0.352	0.329
NAbs					–	0.689***	0.525**
NBT						–	0.532**
PCT							–

ABC = absolute band neutrophil counts; ASC = absolute segmented neutrophil counts; PP = percent of phagocytosis; PI = phagocytic index; NAbs = naturally occurring antibodies; NBT = nitro blue tetrazolium chloride test for detection of hydrogen peroxide production (H_2O_2); PCT = procalcitonin

*P<0.05; **P<0.01; ***P<0.001

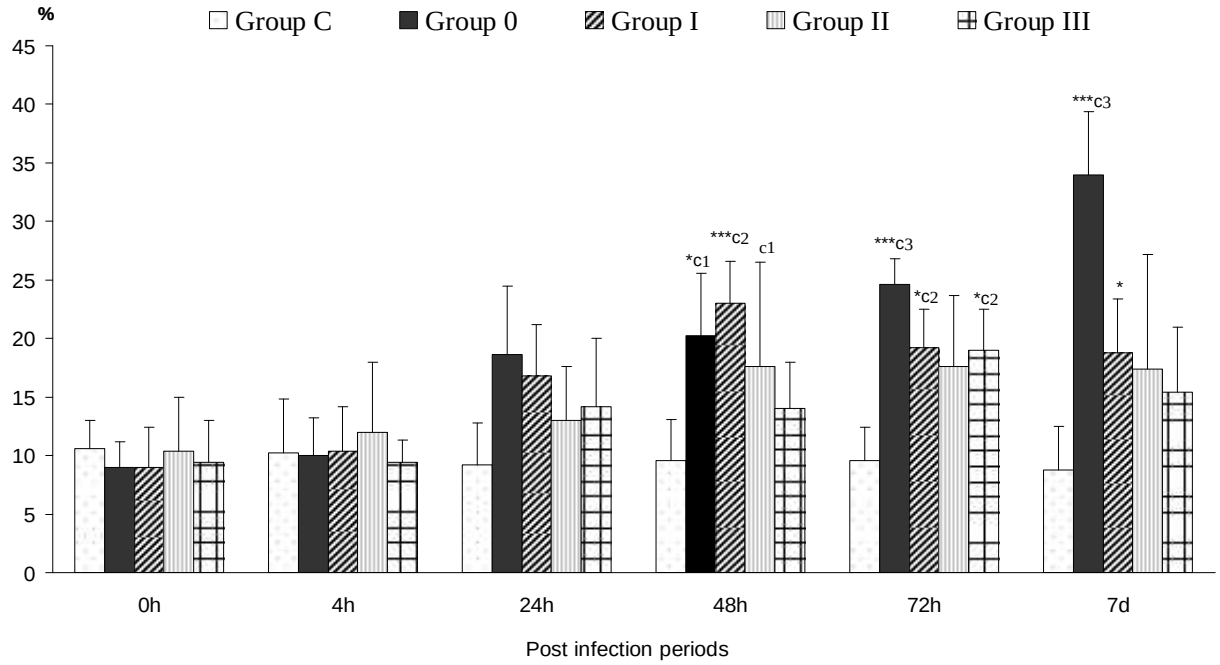

Fig. 1. Changes in hydrogen peroxide producing neutrophils (percentage) in healthy dogs (Group C), dogs infected with *Pseudomonas aeruginosa* (Group 0) and infected dogs treated with: enrofloxacin (Group I); feverfew (Group II) and feverfew+enrofloxacin (Group III). Values are means ± SD of five dogs in each group. Within-group statistical significance: *P<0.05; **P<0.01; ***P<0.001 vs 0 h; between-group statistical significance: c1: P<0.05; c2: P<0.01; c3: P<0.001 – vs control Group C.

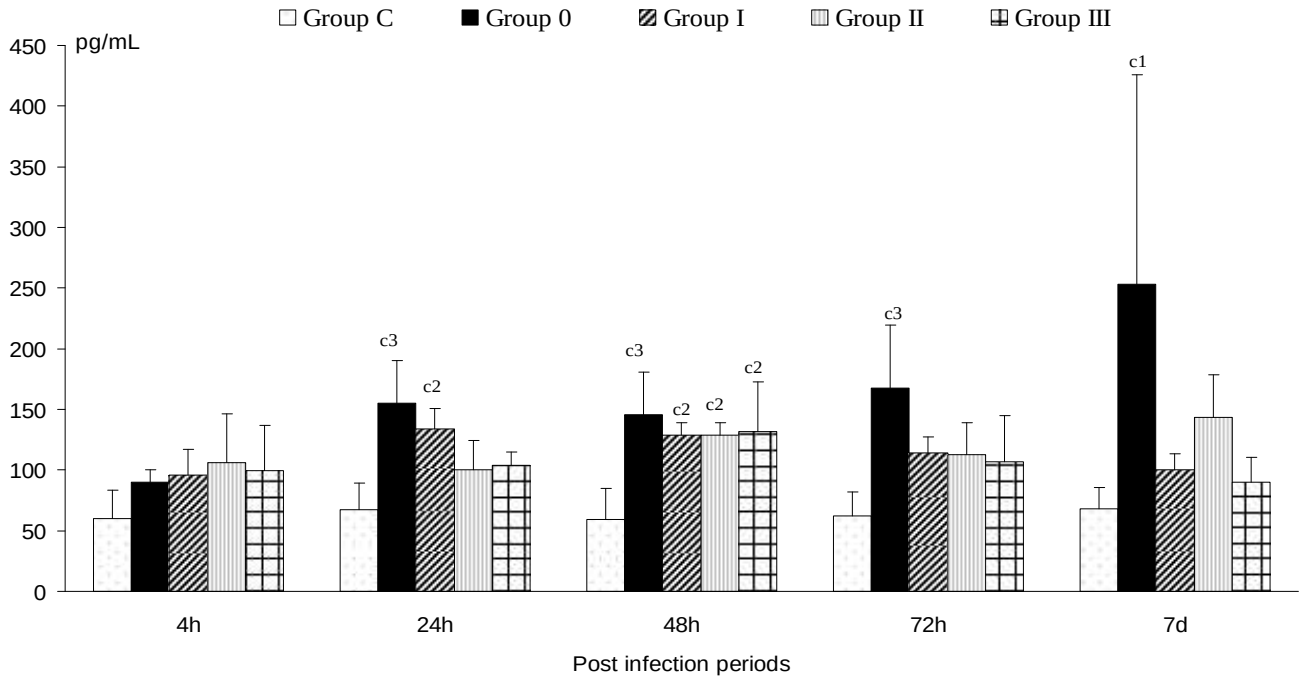

Fig. 2. Changes in serum concentrations of procalcitonin (PCT) (pg/mL) in healthy dogs (Group C), dogs infected with *Pseudomonas aeruginosa* (Group 0) and infected dogs treated with: enrofloxacin (Group I); feverfew (Group II) and feverfew+enrofloxacin (Group III). Values are means ± SD of five dogs in each group. Statistical significance: c1: P<0.05; c2: P<0.01; c3: P<0.001 – vs control Group C.

DISCUSSION

The heterogenous nature of disorders induced by the opportunistic pathogen *P. aeruginosa* combined with non-specific mechanisms of defense triggered at the early stage of the infection impedes the elucidation of key targets of therapy. The experimental canine *P. aeruginosa* infection permitted to eliminate the effects of a number of factors influencing the infection process – inoculum density, site of inoculation, sex, feeding, circadian rhythms, environmental and light conditions. Moreover, it allowed us a better assessment of the dynamics of non-specific mechanisms of defense, whose efficient function is a must for stopping the infection at an earliest stage.

Neutrophils are an essential component of the innate and adaptive immunity (Mantovani *et al.,* 2011) and are critical to keeping us healthy (Nauseef and Borregaard, 2014). Transmigration of these cells from the bloodstream to the site of infection is a key innate immune response against invading pathogens. Therefore, the behaviour of these blood cells in *P. aeruginosa* infection is particularly interesting. According to our experiments, the dogs from Group 0 responded with increase in ABC from post infection hour 48 to day 7 (Table 1). This could be a result of the strong inflammatory response against *P. aeruginosa* (Lovewell *et al.,* 2014). The antibiotic therapy (Group I) was also accompanied by increased ABC and ASC (Table 1). This increases the therapeutic success of enrofloxacin because its accumulation in WBC might increase the concentration of drug at the site of infection (Boothe *et al.,* 2009). The co-administration of antibiotic and phytopreparation (Group III) managed to put these parameters under control because ANC of dogs from Group III varied within the reference range of the species. According to our results, the neutrophil phagocytosis in dogs from Group 0, I, II, III was lower than that in Group C (Table 2). The decrease was most obvious on hour 24 in dogs from Group 0 and dogs receiving feverfew (Group II). This did not correspond to detected high blood neutrophil counts in these animals. It is supposed that this probably reflects the effects of *P. aeruginosa*. This pathogen impairs serum opsonins (both complement and immunoglobulin), the cell receptors, disturbs the cell communication and suppresses the phagocytosis, which are critical in the innate immune response (Lovewell *et al.,* 2014; Alhazmi, 2015). The fact that the number of complement receptors expressed on band neutrophils was lower than on segmented neutrophils indicates that the functional capacity of young forms was not complete (Laarman *et al.,* 2012). This assumption is supported by data in dogs from Group II, exhibiting the highest ABC (Table 1) along with lowest values of phagocytosis (Table 2). The findings indicated that the effects of *P. aeruginosa* were superimposed on effects of parthenolide, which controls the inflammation (Pareek *et*

al., 2011). Smith and Liu (2001) indicated that feverfew extracts inhibit adhesion molecules. The modulation of the leukocyte adhesion expression may be an additional mechanism by which the phytopreparation mediates anti-inflammatory effects. This influence affects not only the inflammatory response, but also another key nonspecific defense mechanism – phagocytosis, NAbs. During physiologic conditions serum contains naturally occurring antibodies, which may play a role in the neutralisation of microbes consequently to their cross-reactivity to microbial antigens (Kaveri, 2012). Natural immunoglobulin G is reported to mediate bacterial clearance during infection/inflammation conditions (Panda and Ding, 2015). In our study, high levels of NAbs were observed in untreated dogs (Group 0), while all treated dogs (Group I, II, III) showed no statistically significant differences vs the negative controls (Table 2). Therefore, effective therapy strategies have to be *in vivo* developed and adjusted without interfering with the host-defense functions. Although PP appears to be disrupted, the capability of neutrophils to ingest microorganisms (PI) (Table 2), as well as to produce H_2O_2 (Fig. 1) was preserved. The fact that neutrophils of dogs from Group 0 produced statistically significantly more H_2O_2 than dogs from Group C over a long period of time (hour 48 – day 7) evidenced the steady-state of H_2O_2. Somprasong *et al.* (2012) commented that H_2O_2 production can be efficiently converted back to oxygen by bacterial catalase which can be used by bacteria, whereas Luckett *et al.* (2012) discussed another defense strategy of the bacterial pathogen, related to its ability to use L-arginine and thus, to block the synthesis of peroxynitrite by phagocytes. The field *P. aeruginosa* strain used in this study was catalase-positive, suggesting that the bacterium could manage easily sharp changes in H_2O_2 production. This isolate is sensitive to enrofloxacin. In infected dogs treated with this drug (Group I), H_2O_2 production was high on hour 48 when the antibiotic therapy started (Fig. 1). This is in agreement with the data of Tall and Veerareddy (2011), demonstrating that fluoroquinolones provoke generation of free radicals (Zhang *et al.,* 2011). It is interesting that this change in H_2O_2 was registered only at the beginning of enrofloxacin application, and thereafter the antibiotic achieved an adjusting effect as shown by the statistically significantly lower H_2O_2 production in Group I vs Group 0 until the end of the study (Fig. 1). Barski *et al.* (2011) investigated the influence of enrofloxacin on the activity of antioxidant enzymes in rats and found that the administration of the antibiotic at applied doses did not evoke oxidative action, especially in the first period after exposure. Analysing the therapeutic potential of parthenolide, George *et al.* (2012) affirmed that it had anti-inflammatory and antioxidant activities. Its application in dogs from Group II (Fig. 1) confirmed antioxidant ability, because H_2O_2 production remained low and increased only after cessation of parthenolide intake. The best correcting effect with respect to H_2O_2 production was attained by co-administration of enrofloxacin and parthenolide (Group III). Despite advances in diagnostic tools, timely identification of bacterial infections remains challenging, often leading to antibiotic misuse with serious health and economic consequences. The time interval to the 72nd hour of infection is the therapeutic window to undertake efficient action and to determine the possible outcome. Therefore, the early clinical evaluation is essential for the adequate clinical decision making (Dupuy *et al.,* 2013). Gilbert (2010) indicated that PCT concentrations can assist with the interpretation of the clinical significance of the results of standard microbiologic methods used to detect bacteria. Our findings demonstrated that serum PCT values in infected untreated dogs increased progressively from the 24th hour to the 7th day (Fig. 2). The treatment with enrofloxacin, parthenolide or with both preparations was accompanied with substantial PCT reduction until day 3 (Fig. 2). Also, the changes in serum PCT concentrations on day 2 and 3 in all treated groups were comparable denoting that PCT should not be the sole criterion for deciding about the administration of antibacterial treatment.

The observed correlations between PCT – a marker of bacterial infection and blood markers – ABC, ASC, neutrophil phagocytosis, oxidative burst activity demonstrated that they could be used in combination as biomarkers to detect the progression of bacterial infection and can help therapeutic decision-making in case of *P. aeruginosa* infection.

Conclusion: The treatment of experimental *P. aeruginosa* infection in dogs with parthenolide (active component of the plant *Tanacetum parthenium*) and enrofloxacin regulated the observed changes in ANC, NAbs, reduced serum PCT concentrations and decreased H_2O_2 production of neutrophils without correction of phagocytosis depression. The latter was also observed after application of feverfew only between post infection hour 4 to day 6. The phytopreparation showed antioxidant activity as shown by lower H_2O_2 production, but additional *in vivo* studies are needed to reveal the full antioxidant and antimicrobial therapeutic potential of the phytopreparation in dogs with *P. aeruginosa* infection.

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