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# Serum biomarkers of oxidative stress in cats with feline infectious peritonitis

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ABSTRACT

The purpose of this study was to elucidate the possible presence of oxidative stress in cats naturally affected by feline infectious peritonitis (FIP) by investigating two antioxidant biomarkers in serum: paraoxonase-1 (PON1) and total antioxidant capacity (TAC). PON1 was measured by spectrophotometric assays using three different substrates: *p*-nitrophenyl acetate (pNA), phenyl acetate (PA) and 5-thiobutil butyrolactone (TBBL), in order to evaluate possible differences between them. The PA and TBBL assays for PON1 and the assay for TAC were validated, providing acceptable precision and linearity although PA and TAC assays showed limit of detection higher than the values found in some cats with FIP. Cats with FIP and other inflammatory conditions showed lower PON1 values compared with a group of healthy cats with the three assays used, and cats with FIP showed significant decreased TAC concentrations. This study demonstrated the existence of oxidative stress in cats with FIP.


## 1. Introduction

Oxidative stress can be defined as an imbalance between the oxidant and antioxidant system, with an advantage toward the oxidant system (Suresh et al., 2009). In this situation the organism is unable to detoxify reactive oxygen species, which accumulate, producing a harmful effect on the functional and structural integrity of biological tissues (Yilmaz, 2012). Cats seem to be more susceptible to oxidative stress and damage, probably influenced by the particular spleen structure of this species (Christopher et al., 1995; Harvey and Kaneko, 1977), and a situation of oxidative stress has been demonstrated in various diseases in this species such as diabetes mellitus (Webb and Falkowski, 2009), chronic renal failure (Keegan and Webb, 2010) and feline immunodeficiency virus (FIV) infection (Webb et al., 2008). However, to the author's knowledge there are no studies about oxidative stress in feline infectious peritonitis (FIP), a viral disease resulting from feline coronavirus (FCoV) infection.

Inflammation plays a major role in FIP infection, since a major inflammatory response is presented during the course of FIP which is involved in the pathogenesis, producing fibrinous serositis, with accumulations of highly proteinaceous fluid within body cavities, disseminated pyogranulomatous formation, hypergammaglobulinemia, and the development of immune complexes (Gunn-Moore et al., 1998; Pedersen, 2014a). Increases in acute phase proteins (APPs), which are markers of inflammation, such as alpha-1 glycoprotein or serum amyloid A can be used as a diagnostic aid in this disease. Knowing the relation between inflammation and oxidative stress (Montorfano et al., 2014), it could be postulated that oxidative stress could be present in cats affected by FIP.

Several enzymes and non-enzymatic molecules are included within the antioxidant system (Delmas-Beauvieux et al., 1996). Paraoxonase 1 (PON1) is a serum enzyme that has a protective role against oxidation (James, 2007). In humans, a reduction of PON1 activity has been reported in several pathologic conditions, including bacterial and viral infections (Farid and Horii, 2012). In addition, PON1 is associated with inflammation, being considered as a negative acute phase protein in several species. Feingold et al. (1998) reported a reduced hepatic synthesis of this enzyme in hamsters during the acute phase response. Decreases have been also described in cattle (Bionaz et al., 2007), laboratory animals (Franco-Pons et al., 2008), humans (Novak et al., 2010), horses (Turk et al., 2011) and dogs (Tvarijonaviciute et al., 2012a, 2012b). Owing to the wide spectrum of species on which PON1 is reduced in inflammation, to study this molecule also in cats may be important.

Lactones are considered as the natural substrates of PON1 (Billecke et al., 2000), and other artificial substrates can also be used for measurement of this enzyme as paraoxon, phenyl acetate (PA)

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or *p*-nitrophenyl acetate (pNA; Ceron et al., 2014). However, use of paraoxon as substrate would not be optimal due to its toxicity (Camps et al., 2009) and the possibility of analyzer contamination (Mogarekar and Chawhan, 2013). Divergences in the diagnostic performance between different substrates have been described in certain diseases (Dantoine et al., 1998; Keskin et al., 2009). Therefore, comparative studies in which various substrates are used for PON1 measurements would be recommended when this enzyme is evaluated in a new disease.

Serum total antioxidant capacity (TAC) considers the cumulative effect of all antioxidants present in the blood (Nagy et al., 2006) and provides an integrated index of the oxidative status (Ghiselli et al., 2000). The most widely used colorimetric methods are based on oxidation of a colorless molecule, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), to a blue–green $ABTS^{+}$ (Erel, 2004). Decreased TAC has been reported in human patients with HIV infection and was negatively correlated with lipid peroxidation, suggesting the presence of oxidative stress in these individuals (Suresh et al., 2009).

We hypothesize that oxidative stress could be present in the cats affected by FIP, probably influenced by the major inflammatory reaction which is associated with this disease, and therefore changes in serum oxidative biomarkers may be present. To test this hypothesis, serum PON1 activity and TAC in a group of cats naturally affected by FIP were measured and compared with a group of healthy cats and cats with other inflammatory diseases. PON1 activity was measured using three different substrates: pNA, PA and 5-thiobutil butyrolactone (TBBL) to evaluate the possible differences in the values of PON1 in FIP depending on the substrate. Assays for measurement of PON1 using PA and TBBL, and an assay for TAC determination, were validated for use in cats.

## 2. Materials and methods

### *2.1. Animals*

In this retrospective study, the serum samples from cats were selected from the authors' veterinary hospitals database (2011–2012). The serum samples corresponded to diagnoses made based on physical examination, hematological and biochemistry evaluations, urinalyses, radiography and ultrasonography, cytology, serology and immunohistochemistry, depending on each clinical case. Based on the results of the different diagnostic approaches, the serum samples were selected and separated into 3 groups.

Serum of 18 clinically healthy cats presented for routine health screening examinations was the control group. These cats had no history of illness and no clinical signs on physical examination; all were serologically negative for FCoV infection using indirect fluorescence antibody (VMRD), FIV and feline leukaemia virus (FeLV) infections by using commercially available ELISA tests (ViraCHEK, Synbiotics). Serology was considered positive when titers were >1/80 in case of FeLV and FIV tests and when titers were >1/1600 in case of FCoV tests. These cats did not show any clinical sign compatible with FIP 1 year after sample collection.

The second group consisted of serum from 19 cats naturally infected with FCoV that presented clinical signs consistent with FIP. All these cats had negative serology to FIV and FeLV. Hematological changes included low packed cell volume in 7/19 cats, neutrophilia in 8/19 cats and lymphopenia in 12/19 cats. FIP diagnosis was confirmed in all 19 cats by necropsy, histology and immunohistochemistry (Licitra et al., 2013). Fourteen cats had effusive FIP and 5 cats had the non-effusive or dry form.

The third group consisted of serum from cats with other inflammatory diseases. This group was comprised of 13 cats that were serologically negative for FCoV, FeLV and FIV infections, but had high serum amyloid A (SAA) concentrations (median 42.9 µg/ml; interquartile range 25.8–53.7 µg/ml) consistent with inflammatory disease. Diagnoses in this group included feline lower urinary tract disease, bone fracture, cholangiohepatitis ($n = 2$ each), chronic renal insufficiency, aseptic pneumonia, gastroenteritis, cranial trauma, dehiscence of a surgical stitches, chronic interstitial nephritis and pyometra ($n = 1$ each). One cat with cholangiohepatitis, the cat with chronic renal insufficiency and the cat with chronic interstitial nephritis died in a period of less than 1 year after collecting the samples and post mortem examination rule out the presence of FIP. The cats that survived did not presented signs compatible with FIP 1 year after collecting the samples.

The serum samples were kept at −80 °C until analyses for PON1, TAC and SAA. This study was approved by the Ethics Committee of the University of Murcia (Spain).

### *2.2. PON1 analyses*

#### *2.2.1. Serum PON1 activity measured with pNA*

This activity was measured following a previously described method (Tvarijonaviciute et al., 2012b). Three hundred microliters of the working reagent consisted of 50 mM Tris (Tris [hydroxymethyl] aminomethane, Sigma-Aldrich), pH 8.0, with 1.0 mM $CaCl_2$ (calcium chloride dihydrate, Sigma-Aldrich) was added together with 2 µl of the serum sample. After an incubation period of 325 s at 37 °C, 72 µl of the start reagent consisting of 2.5 mM pNA (Sigma-Aldrich) in water was added. The rate of formation of *p*-nitrophenol was determined at 405 nm after 250 s in an automated chemistry analyzer (Olympus 2700). The nonenzymatic hydrolysis of pNA, which was based on the hydrolysis rate in the absence of serum, was subtracted from the total hydrolysis rate. The activity, expressed in U/ml, was based on the molar absorptivity (14,000/M/cm) of *p*-nitrophenol at 405 nm. This method has been previously validated in cats (Tvarijonaviciute et al., 2012c).

#### *2.2.2. Serum PON1 activity measured with PA*

In this method, PON1 activity was analyzed by measuring the hydrolysis of PA into phenol as described elsewhere (van Himbergen et al., 2005). The assay was performed in a 96-well microplate. The sample buffer consisted of 50 mM Tris and 1 mM $CaCl_2$ (pH 8.0). The serum sample was diluted in sample buffer to 1:40 ratio, and 5 µl of the diluted sample was added to the wells. Then, 200 µl of the freshly made substrate reagent containing 1 mM PA (Sigma-Aldrich) in sample buffer was added. The reaction was monitored for 5 min at 260 nm and 37 °C in a microplate reader (PowerWave XS, Bio-Tek Instruments). The nonenzymatic hydrolysis of PA, which was based on the hydrolysis rate in the absence of serum, was subtracted from the total hydrolysis rate. PON1 activity was expressed as U/ml of serum. The molar extinction coefficient used to calculate the rate of hydrolysis was 1310/M/cm.

#### *2.2.3. Serum PON1 activity measured with 5-thiobutil butyrolactone (TBBL)*

The method involves the use of a chromogenic lactone that structurally resembles the proposed natural lipolactone substrates (Marsillach et al., 2009). The assay was performed in a 96-well microplate. The sample buffer consisted of 50 mM Tris and 1 mM $CaCl_2$ (pH 8.0). The method comprised of four pippeting steps: 1 µl of a chromophore solution containing 100 mM 5,5′-dithio-bis-2-nitrobenzoic acid (Sigma-Aldrich Co) in dimethyl sulfoxide (Sigma-Aldrich Co) was added to the wells, then followed by 45 µl of 4% acetonitrile (Mulrisolvent HPLC grade ACS, Sharlau Chemie SA, Sentmenat, Spain) solution in sample buffer. In the third step, 55 µl of diluted serum sample at 1:200 ratio in sample buffer was added. Finally, 100 µl of the freshly made substrate containing 0.4 mM TBBL (provided by Dr. Khersonsky, Weizmann Institute of Science, Israel) in sample buffer was added. Two minutes after TBBL addition, the reaction was monitored at 412 nm in an automated microplate reader (PowerWave XS) at 37 °C. The nonenzymatic hydrolysis of TBBL, which was based on the hydrolysis rate in the absence of serum, was subtracted from the total hydrolysis rate. PON1 activity was expressed as U/ml of serum, in which 1 unit equals 1 mmol of TBBL hydrolyzed/min. The molar extinction coefficient used to calculate the rate of hydrolysis was 7000/M/cm.

### 2.3. TAC measurement

The method described by Erel (2004) was validated for use in cats. In this method, the blue–green colored oxidized 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) is reduced to a colorless molecule, and the change in color is spectrophotometrically monitored. Reagent 1 was consisted of acetate buffer solution, pH 5.8, including 0.4 M $CH_3COONa$ (Sigma-Aldrich) and 0.36% glacial acetic acid (Sigma-Aldrich). Reagent 2 was consisted of acetate buffer, pH 3.6, containing 2 mM $CH_3COONa$, 0.15% glacial acetic acid, 0.009% hydrogen peroxide ($H_2O_2$, Panreac Química SA) and 10 mM ABTS diammonium salt (Sigma-Aldrich). Two hundred fifty microliters of reagent 1 and 12 µl of serum sample were mixed and incubated at 37 °C for 150 s. Then, 25 µl of reagent 2 was added. Change in absorbance was monitored at 600 nm after 525 s. The method was adapted to an automated analyzer (Olympus 2700).

### 2.4. Serum amyloid A (SAA) measurement

The presence of inflammation was assessed by measuring the serum acute phase protein SAA concentration. SAA was measured using a commercially available immunoturbidimetric method (SAATIA; LZ-SAA, Eiken Chemical Co., Tokyo, Japan), adapted to an automated analyzer (Olympus 2700). This method has been previously validated for feline specimens (Kajikawa et al., 1999).

### 2.5. Analytical validation for PON1 measurements with PA and TBBL, and TAC measurements

Since these methods have not been previously validated for feline specimens, an analytical validation was performed. Precision was determined by calculating the intra-assay coefficient of variation (CV) of two pooled sera with different PON1 and TAC concentrations. The pool with low PON1 activities and low TAC concentration was performed by mixing the same volume of serum from 10 diseased animals which were randomly chosen and the pool with expected high PON1 activities and high TAC concentration was prepared by mixing sera from 10 healthy animals. Each pool was analyzed six times in a single assay run. The inter-assay CV was determined by analyzing the same pools in six separate runs on different days. Sera were frozen in aliquots, and only the vials needed for each run were used, to avoid possible changes due to repetitive thawing and freezing. Linearity was evaluated using the method of linearity under dilution. The pool serum with high values of PON1 and TAC was diluted on a two-fold basis with sample buffer and assayed by triplicate. The results were compared with those expected by linear regression analysis. The limit of detection of the assay (the lowest concentration of each analyte that could be distinguished from a specimen with a value of zero) was calculated as the mean plus 2 standard deviations for data generated from 20 replicate determinations of the zero standard (sample buffer).

### 2.6. Statistical analyses

Routine descriptive statistic parameters were calculated by routine descriptive statistical procedures. The Kolmogorov–Smirnov test was performed to assess the normality of data obtained from the healthy and diseased cats. Those data giving a non-parametric distribution were log-transformed prior to statistical analysis. One-way analysis of variance and Tukey post-hoc test were used to determine if the values obtained from the healthy and diseased animals were statistically different. The Pearson correlation coefficient was calculated to assess correlation between PON1 and TAC with SAA. The significance level used in each case was $P < 0.05$. All statistical analyses were calculated using spreadsheet (Excel 2000, Microsoft) and Graph Pad Software Inc (GraphPad Prism, version 5 for Windows, Graph Pad Software).

**Table 1**
Mean, standard deviation (SD) and coefficients of variation (CV) obtained for paraoxonase and total antioxidant capacity in pooled cat sera.

| | | Intra-assay | | Inter-assay | |
|---|---|---|---|---|---|
| | | Mean (SD) | CV | Mean (SD) | CV |
| PA (U/ml) | Pool 1 | 39.21 (1.44) | 3.68 | 33.34 (0.92) | 2.76 |
| | Pool 2 | 11.01 (0.67) | 6.11 | 10.40 (0.71) | 6.81 |
| TBBL (U/ml) | Pool 1 | 4.16 (0.09) | 2.23 | 3.99 (0.22) | 5.60 |
| | Pool 2 | 1.21 (0.11) | 9.35 | 1.04 (0.16) | 15.11 |
| TAC (mmol/l) | Pool 1 | 0.64 (0.01) | 1.50 | 0.60 (0.03) | 4.80 |
| | Pool 2 | 0.15 (0.01) | 5.70 | 0.15 (0.02) | 11.30 |

PA, paraoxonase measured with phenyl acetate; TBBL, paraoxonase measured with 5-thiobutil butyrolactone; TAC, total antioxidant capacity.

## 3. Results

### 3.1. Analytical validation of PON1 measurements with PA and TBBL, and TAC measurements

The intra- and inter-assay CVs obtained for PON1 measurement with PA and TBBL and for TAC analysis are shown in Table 1. Intra-assay CVs were lower than 10% in all cases. Inter-assay CVs were lower than 7%, 16% and 12% for PA, TBBL and TAC, respectively. Coefficient of linear regression higher than 0.98 was observed when pooled serum was diluted and analyzed with any of the validated methods (Fig. 1). The calculated limits of detection were 0.64 U/ml, 0.04 U/ml and 0.02 mmol/l for PA, TBBL and TAC, respectively.

### 3.2. Comparison between healthy and diseased cats

Table 2 shows PON1, TAC and SAA values in the cats included in this study. A significantly lower PON1 activity was observed with all substrates in cats with FIP or with other inflammatory dis-

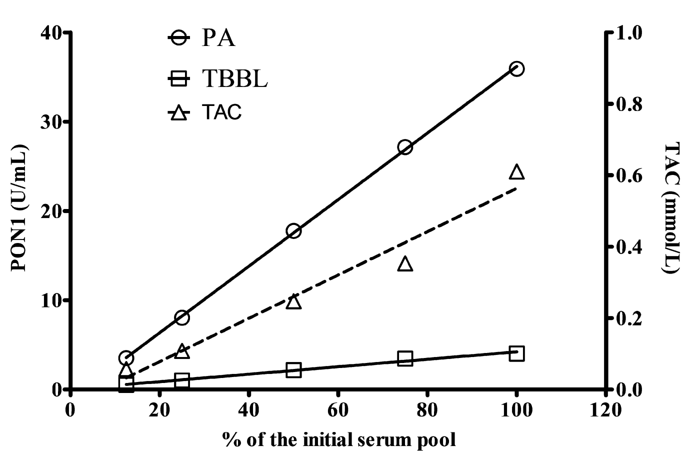


**Fig. 1.** Linear regression analysis obtained after analysis of a pool of serum diluted on a two-fold basis with sample buffer. Dilutions were assayed by triplicate for paraoxonase (PON1) with the substrates phenyl acetate (PA) and 5-thiobutil butyrolactone (TBBL), and total antioxidant capacity (TAC). Each point represents the mean value of the triplicates.

**Table 2**
Paraoxonase activity (measured with three different substrates), total antioxidant capacity and serum amyloid A concentration in healthy cats, cats with feline infectious peritonitis and cats with non-viral inflammation. Results are expressed as median value, 25–75% centiles are within parenthesis, minimum–maximum values are within brackets.

| | pNA (U/ml) | PA (U/ml) | TBBL (U/ml) | TAC (mmol/l) | SAA (mg/l) |
|---|---|---|---|---|---|
| Healthy | 3.45 | 25.30 | 3.39 | 0.42 | 0.15 |
| | (2.58–4.58) | (18.38–34.31) | (2.43–4.15) | (0.32–0.47) | (0.10–0.30) |
| | [1.30–5.80] | [4.40–58.50] | [0.88–4.94] | [0.10–0.61] | [0.10–0.40] |
| FIP | 1.90** | 9.61*** | 1.62*** | 0.26** | 107.10*** |
| | (1.33–2.48) | (4.43–12.34) | (0.73–2.16) | (0.00–0.30) | (38.00–141.80) |
| | [0.90–4.50] | [3.75–22.38] | [0.43–3.30] | [0.02–0.47] | [4.00–260.10] |
| Inflamm. | 1.30*** | 9.20*** | 1.79* | 0.34 | 42.90*** |
| | (0.45–2.35) | (3.75–17.80) | (1.16–2.47) | (0.18–0.46) | (24.85–55.00) |
| | [0.30–4.20] | [0.64–27.76] | [0.42–3.43] | [0.02–0.54] | [17.00–73.90] |

pNA, *p*-nitrophenyl acetate; PA, phenyl acetate; TBBL, 5-thiobutil butyrolactone; TAC, total antioxidant capacity; SAA, serum amyloid A; FIP, feline infectious peritonitis; Inflamm, cats with other inflammatory diseases.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

eases, compared with healthy cats. TAC was significantly lower in cats with FIP compared with healthy cats and cats with other inflammatory conditions. The SAA concentration was significantly higher in cats with FIP and with other inflammatory diseases than in healthy animals. The SAA concentration was significantly higher in cats with FIP than in cats with other inflammatory diseases.

In Table 3, the values of the different analytes in cats with effusive and non-effusive forms appear. Cats with effusive forms showed significantly lower values of PON1 and TAC and higher values of SAA compared with cats with non-effusive forms.

No significant correlation was observed between PON1 measured with different substrates and TAC. All PON1 assays and TAC measurement showed a significant but weak negative correlation with SAA concentration (Table 4).

## 4. Discussion

In this report, assays for PON1 activity with three different substrates (pNA, PA and TBBL) and TAC were used. PON1 assays using PA and TBBL and the assay for TAC determination were first validated for feline samples. In the PON1 assay, both methods using PA and TBBL showed precise results and were able to measure PON1 in a linear manner, similar to the results previously reported in dogs (Tvarijonaviciute et al., 2012b). Although one cat with FIP showed a PON1 value below the limit of detection when PA was used. The TAC assay was precise and linear with values higher than the limit of detection of the assay. However, 5 cats with FIP and one cat with other inflammatory condition showed TAC values under the calculated limits of detection. The existence of values lower than the limit of the detection should be considered as a limitation for the PON1 assay using PA as substrate and for the TAC assay, especially in cases of FIP or other inflammatory conditions where low values are expected.

To the authors' knowledge, this is the first report measuring PON1 and TAC in cats with FIP and in inflammatory conditions. A significant decrease in PON1 activity with the three substrates was observed in both groups of diseased cats. The only report found about PON1 in cats studied the possible oxidative stress associated with obesity (Tvarijonaviciute et al., 2012c). There are several reasons that could explain a decrease in PON1 activity in cats with FIP. One of these reasons is oxidative stress, corroborated by the low TAC concentration in these cats. Oxidative stress is known to be involved in the pathogenesis of some human viruses. For example, hepatitis C virus infection is associated with the accumulation of reactive

**Table 3**
Paraoxonase activity (measured with three different substrates), total antioxidant capacity and serum amyloid A concentration cats with feline infectious peritonitis in the effusive form and in the dry form. Results are expressed as median value, 25–75% centiles are within parenthesis, minimum–maximum values are within brackets.

| | pNA (U/ml) | PA (U/ml) | TBBL (U/ml) | TAC (mmol/l) | SAA (mg/l) |
|---|---|---|---|---|---|
| Effusive | 1.50** | 7.78* | 0.93** | 0.22* | 107.60** |
| | (1.20–2.30) | (4.38–11.59) | (0.67–1.70) | (0.02–0.25) | (58.85–191.90) |
| | [0.90–3.00] | [3.75–12.82] | [0.43–2.07] | [0.02–0.30] | [27.90–260.10] |
| Dry | 2.90 | 14.95 | 2.20 | 0.28 | 41.80 |
| | (0.45–2.35) | (7.03–19.00) | (2.16–2.90) | (0.26–0.34) | (13.70–54.50) |
| | [2.20–4.50] | [3.99–22.38] | [2.16–3.30] | [0.26–0.47] | [4.00–60.10] |

pNA, *p*-nitrophenyl acetate; PA, phenyl acetate; TBBL, 5-thiobutil butyrolactone; TAC, total antioxidant capacity; SAA, serum amyloid A.
* $P < 0.05$.
** $P < 0.01$.

**Table 4**
Pearson correlation coefficients between paraoxonase assays with serum amyloid A and total antioxidant capacity.

| | pNA (U/ml) | PA (U/ml) | TBBL (U/ml) | TAC (mmol/l) |
|---|---|---|---|---|
| Pearson correlation coefficient with TAC | 0.226 | 0.130 | −0.036 | |
| Pearson correlation coefficient with SAA | −0.509*** | −0.594*** | −0.377* | −0.376** |

pNA, *p*-nitrophenyl acetate; PA, phenyl acetate; TBBL, 5-thiobutil butyrolactone; TAC, total antioxidant capacity; SAA, serum amyloid A.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

oxygen species and reduced glutathione activity (Paracha et al., 2013) and HIV infection decreases PON1 activity (Parra et al., 2007, 2010) and TAC (Suresh et al., 2009). The presence of inflammation and increase in inflammatory cytokines that appear in cats with FIP (Regan et al., 2009) could also explain the decreased PON1 activity, since it is considered part of the innate immune system (Chun et al., 2004) and is a negative acute phase protein that decreases during inflammation (Novak et al., 2010; Tvarijonaviciute et al., 2012a), as it can be seen in our study in the group of cats with an inflammatory condition. Inflammation results in the loss of PON1 from HDL-cholesterol molecules, to be replaced by SAA (Kappelle et al., 2011). This could explain the increase in SAA and the decrease in PON1 demonstrated in cats with FIP in this study, indicating a pronounced inflammatory response, and the significant (although weak) negative correlation observed between SAA and PON1.

SAA analysis was performed in order to assess the presence of an inflammatory response in all the cats used in this study. Although AGP has been reported to be the APP of choice in cats with FIP we chose SAA since it is an automated method, so its measurement is easier than AGP, which determination is currently made in most lab by radial immune diffusion technique. Besides, it has been demonstrated that SAA shows similar response in FIP cats than AGP does (Giordano et al., 2004).

Although the three substrates used for PON1 assays provided similar results decreasing in cats with FIP, from the technical point of view pNA could be considered as an ideal substrate. Compared with PA, it is less toxic and has a lower limit of quantification (Tvarijonaviciute et al., 2012b). In addition, pNA assay can also be automated, thereby allowing higher specimen throughput and avoiding pipetting errors, which results in higher precision than TBBL which exceeded the recommended limit of 15% for inter-assay measurements (Guidance for Industry: Bioanalytical Method Validation, US, 2001). In a study performed in dogs, higher significance in the differences between dogs with oxidative damage and healthy dogs was found with pNA compared with PA or TBBL (Tvarijonaviciute et al., 2012b). Another important limitation for TBBL use is that this substrate is not commercially available.

FIP cats with the wet-effusive form showed lower values of PON1 and TAC, indicating a higher oxidative stress compared with cats with the dry form. This could be related with the poorer outcome that FIP cats with effusive form have compared with the dry form (Pedersen, 2014b), and although further studies should be made to elucidate it, oxidative markers could help to establish a prognostic in FIP cases. The knowledge of the pathogenesis of FIP remains at a very basic level (Pedersen, 2014a), and no studies about the influence of oxidative stress in the physiopathology of FIP has been undertaken. However, in other viral infectious such as HIV, changes in the various antioxidant defenses may also be associated with progression of the disease (Treitinger et al., 2000).

One point to discuss, which could be considered as a limitation of this paper, is the high cut-off test used for considering a cat as FCoV seropositive (1:1600). This was chosen because coronavirus antibody titers are increasingly suggestive of FIP at higher titers than 1:1600 (Pedersen, 2014b). It could be argued that the use of a lower cut-off (i.e. 1:25) would have allowed to detect more FCoV seropositive cats, however it is considered that coronavirus antibody titers can be misleading in the range of 1:25–1:1600 (Pedersen, 2014b).

## 5. Conclusions

This paper reports by first time the decrease of two antioxidant biomarkers PON1 and TAC in cats with FIP compared with healthy animals. This indicates that a situation of oxidative stress appears in FIP possibly associated with the inflammatory response that occurs in this disease. Further research should be performed to explore the possible practical applications that monitoring the oxidative stress could have in this disease, as well the possibilities of antioxidants having therapeutic or preventative potential.

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