Original Article

Role of paraoxonase-1 (PON-1) as a diagnostic marker for feline infectious peritonitis (FIP)

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Abstract

Feline infectious peritonitis (FIP) is characterized by a huge inflammatory response accompanied by oxidative stress. Paraoxonase-1 (PON-1) is a liver enzyme that circulates in blood bound to high density lipoproteins. Its hydrolytic properties have been associated with an antioxidant activity and its role as negative acute phase reactant has been investigated in people and animals. A paraoxon based method has been validated to measure feline serum PON-1 activity, allowing to observe a negative correlation between PON-1 activity and acute phase proteins concentration. . The aim of this study was to investigate the usefulness of PON-1 as a biomarker able to discriminate FIP from other diseases with similar clinical signs. Of 159 cats enrolled, 71 were healthy, 34 were affected by FIP and 54 were affected by other clinical conditions with signs consistent with FIP. PON-1 activity was lower (P <0.0001) in cats with FIP (median= 26.55 U/L; min – max= 5.40 – 78.20 U/L) compared to healthy (87.5 U/L; 46.60 – 215.50 U/L) and NON FIP cats (57.90 U/L; 3.80 – 122.60 U/L). The receiver operating characteristic curve allowed to determine the threshold that maximizes the performance of PON-1 in predicting FIP: a value of 51.40 U/L yielded 82% sensitivity and specificity and a positive likelihood ratio of 4.65. A value of 24.90 U/L maximizes specificity (97.6%) and increases the likelihood ratio up to 18.38, making PON-1 a good confirmatory test for FIP. Using these thresholds, serum PON-1 activity showed optimal diagnostic performances in discriminating FIP affected cats from cats with other inflammatory conditions.

Keywords: Acute phase protein; Biomarker; Feline infectious peritonitis; Paraoxonase-1

Introduction

Feline infectious peritonitis (FIP) is a systemic fatal disease affecting mostly young cats and sustained by a virulent biotype of the feline coronavirus (FCoV) (Pedersen, 2014a). FCoVs are usually found in the intestinal tract and named "less virulent FCoV" (previously called feline enteric coronavirus, FECV) (Kennedy, 2020). However, during viral replication, a mutated variant, called FIP-associated FCoV (FIPV) may be generated (Pedersen, 2014a). Although both viral biotypes can spread systemically, only the FIPV is able to induce FIP. Depending on the host immune response, clinical presentation of FIP can include effusions in one or several body cavities (effusive or wet form), or granulomatous lesions in different organs (non-effusive or dry form) (Pedersen, 2014a).

The *in vivo* diagnosis of FIP is challenging, especially in dry forms, despite the availability of several diagnostic tests (Tasker, 2018). The definitive diagnosis can only be achieved by demonstrating the presence of intralesional FCoV through immunohistochemistry, usually on *post-mortem* biopsies (Pedersen, 2014b; Tasker, 2018). *In vivo*, the suspicion of FIP relies on signalment, clinical history and laboratory data (Addie et al., 2009; Stranieri et al., 2017; Tasker, 2018). In wet forms, the effusions analyses including macroscopic appearance, cytological features and instrumental findings, can be very helpful for FIP diagnosis (Addie et al., 2009; Giordano et al., 2015; Saunders, 2016; Tasker, 2018). Despite spike gene mutations may be indicative of FIP-associated FCoV, RT-PCR is not able to distinguish the two biotypes (Felten and Hartmann, 2019). However, a positive result may support the clinical suspicion of FIP, while a negative result cannot rule out the disease (Kipar and Meli, 2014; Saunders, 2016; Felten et al., 2017, Stranieri et al., 2018). The increase of alpha-1-acid glycoprotein (AGP) on serum is highly suggestive of FIP but results based on a radial immunodiffusion assay are prone

to subjective interpretation and require at least two days to be generated (Paltrinieri et al., 2007; Giori et al., 2011; Tasker, 2018).

Paraoxonase-1 (PON-1) belongs to a group of enzymes (paraoxonases) and it can metabolize the organophosphate paraoxon. PON-1 is a glycoprotein, synthetized by the liver, that circulates in blood associated with high density lipoproteins (HDL) particles, specifically the apolipoprotein-1 (Furlong et al., 2016; Shunmoogam et al., 2018). During inflammation, especially when associated with a marked oxidative stress, the HDL particles lose the apolipoprotein-1 and many associated enzymes, including PON-1, that are replaced by serum amyloid A and ceruloplasmin (Khovidhunkit et al., 2004; Rossi et al., 2020). Moreover, hepatic synthesis of PON-1 is inhibited during inflammation (Feingold et al., 1998), suggesting that PON-1 acts as a negative acute phase reactant. PON-1 activity decreases during severe inflammatory processes in humans as well as in several animal species, including cats (Giordano et al., 2013; Rossi et al., 2013; Rossi et al., 2020). Cats are more susceptible than other species to oxidative injuries (Feingold et al., 1998). A recent study has demonstrated the intimate relationship between inflammation and oxidative stress during feline infectious diseases (Tecles et al., 2015). Despite lower values of PON-1 activity were expected in cats affected by FIP, in the cited study no differences in PON-1 activity between cats with FIP or other diseases were found. Moreover, PON-1 activity was measured by Tecles and colleagues using a method different from the paraoxon based method, which was recently validated in cats (Rossi et al., 2020). This recent validation study also revealed a negative correlation between serum AGP and PON-1, supporting the role of this enzyme as negative acute phase reactant.

The aim of the present study was to evaluate the potential of PON-1 in discriminating FIP affected cats within a group of animals with clinical signs consistent with the disease and to establish its usefulness as an *in vivo* biomarker.

Materials and methods

Animals and Sample Collection

All the animals were submitted for clinical examinations to the Veterinary Teaching Hospital (University of Milan) or to private practitioners. Both clinically healthy cats and cats with clinical signs consistent with FIP, regardless of the final diagnosis, were enrolled.

Inclusion criteria for the entire caseload were at least the results of the complete blood cell count and a serum biochemistry panel (including creatinine, urea, total protein, alanine aminotransferase, alkaline phosphatase and glucose) and the availability of serum leftover from the routine diagnostic procedures (at least 150 μL). Serum leftover was frozen at -20°C until further analyses.

The cats were divided in two groups: sick group and control group. Inclusion criteria for the control group (CNTR) were a normal physical examination and the absence of laboratory abnormalities. For sick cats, the inclusion criteria were the presence of one or more clinical signs commonly associated with FIP, namely: effusions, hyperthermia, anorexia, weight loss, depression, jaundice, neurological or ocular signs (Tasker, 2018).

Moreover, the sick group was divided in FIP and NON FIP groups. Specifically, the diagnosis of FIP (FIP group) was achieved by positive IHC revealing FCoV antigen within intra-lesional macrophages on *post mortem* biopsies, using the IHC protocol employed in

previous studies (Stranieri et al., 2020). When the *post mortem* examination was not authorized by the owners, the diagnosis of FIP was achieved based on signalment, clinical signs and at least five of the following laboratory changes consistent with FIP (lymphopenia; hyperproteinemia; hypoalbuminemia; low albumin/globulin ratio; electrophoretic peaks in α-2 and γ region; serum AGP higher than 1.5 mg/mL; positive RT-PCR on blood, tissues or effusions and a delta total nucleated cells measured by Sysmex XT-2000iV [ΔTNC] higher than 2.5 on effusions along with consistent cytology) along with the worsening of the clinical conditions that always led to spontaneous death or to humane euthanasia (Paltrinieri et al., 2007; Giordano et al., 2013).

Further, cats belonging to the FIP group were divided according to the clinical form of the disease, namely effusive (WET FIP) and non-effusive FIP (DRY FIP) or according to the type of diagnostic approach, namely FIP confirmed by IHC (CONFIRMED FIP) or FIP suspected based on clinical and clinical-pathological results but not confirmed by IHC (PRESUMPTIVE FIP).

Diseases of cats included in the NON FIP group were diagnosed with diagnostic imaging (ultrasound, MRI, TC) and laboratory data confirming the presence of a specific clinical condition other than FIP.

The samples had been collected for diagnostic purposes according to standard veterinary procedures. Therefore, according to the regulations of our institution, a formal approval of the Institutional Ethical Committee was not required (EC decision 29 Oct 2012, renewed with the protocol n° 02-2016).

Measurement of PON-1 activity

PON-1 activity was measured using a paraoxon based method, already validated in cats (Rossi et al., 2020) on an automated spectrophotometer (Cobas Mira, Roche Diagnostic),

Statistical Methods

Statistical analyses were performed using the software Analyse-it for Microsoft Excel (Analyse-it Software Ltd). Specifically, differences in PON-1 activity among groups (CNTR, FIP and NON FIP) were evaluated using the Kruskall-Wallis test, followed, in case of significant differences, by the Mann-Whitney *U* test.

The comparison between the FIP subgroups (namely WET FIP vs DRY FIP and CONFIRMED FIP vs PRESUMPTIVE FIP) was performed using Mann-Whitney *U* test. PON-1 activity of each FIP subgroup was compared with those of NON FIP and CNTR groups using the Kruskall-Wallis test, followed, in case of significant differences, by the Mann-Whitney *U* test. Statistical significance was set at $P < 0.05$.

Finally, to evaluate the diagnostic power of PON-1 activity in discriminating FIP affected cats, sensitivity and specificity were calculated using standard formulas (Christenson, 2007). Moreover, positive (LR+) and negative (LR-) likelihood ratios were calculated using the following formulae: LR+ = (sensitivity)/(1-specificity) and LR- = (1-sensitivity)/(specificity). A receiver operating characteristic curve (ROC) was built by plotting sensitivity *vs* 1-specificity. The area under the curve (AUC) was calculated and the ROC curve was used to determine clinically relevant thresholds (Gardner and Greiner, 2006).

Results

Group composition

A total of 159 cats was enrolled. Among those, 71 were healthy (CNTR group), 54 were affected by disease other than FIP (NON FIP group) and 34 were affected by FIP (FIP group). Details about cats signalment are reported in Table 1.

In the NON FIP group, 47 cats presented an effusion (19 peritoneal, 30 pleural, 6 pericardial, 8 cats had bi-cavitary effusions). The remaining 7 cats had hyperthermia and lethargy (n = 4) and neurological or ocular signs (n = 3). Most of the cats had a definitive diagnosis of neoplasia (n = 21), followed by cardiogenic effusion (n = 7), chylothorax (n = 6), septic effusion (n = 7), infectious diseases (n = 4), IMHA (n = 2), pleuritis (n = 1), intestinal occlusion (n = 1), traumatism (n = 3) or hepatic lipidosis (n = 2).

The FIP group was composed by 27 cats with effusions (21 peritoneal, 8 pleural, 2 pericardial. Four cats showed bi- or tri-cavitary effusion). Seven cats were affected by the dry from with the presence of neurological or ocular signs (n = 4) or non-specific signs (hyperthermia, jaundice, lethargy and vomiting, n = 3). In 19 cases, the definitive diagnosis was achieved through IHC (CONFIRMED FIP). In the remaining 15 cases, FIP was diagnosed according to laboratory abnormalities highly consistent with FIP, along with the severe clinical conditions that always ended with spontaneous death or euthanasia (PRESUMPTIVE FIP) (Table 2).

Difference in PON-1 activity among CNTR, FIP and NON FIP

PON-1 activity was significantly different among the three groups (P <0.0001). Specifically, PON-1 activity was significantly lower in FIP cats (median= 26.55 U/L; min – max= 5.40 – 78.20 U/L) than in CNTR (87.5 U/L; 46.60 – 215.50 U/L) and NON FIP cats

193 (57.90 U/L; 3.80 – 122.60 U/L) (P <0.0001 in both cases) (Fig. 1). Cats belonging to the NON
194 FIP group had values of PON-1 activity significantly lower than the CNTR cats (P <0.0001).
195
196 *Comparison between presumptive FIP and confirmed FIP*
197 The values recorded in the PRESUMPTIVE FIP subgroup (30.60 U/L; 5.40 – 68.10
198 U/L) were not significantly different from the CONFIRMED FIP subgroup (25.70 U/L; 6.30 –
199 78.20 U/L) (P = 0.61). Conversely, the results of both subgroups were significantly lower
200 compared with NON FIP and CNTR groups (P <0.0001) (Fig.2).
201
202 *Comparison between effusive and non-effusive FIP*
203 The cats with effusive FIP (WET FIP) had a lower PON-1 activity (23.20 U/L; 5.40 – 71.50
204 U/L) compared with those with DRY FIP (48.00 U/L; 15.10 – 78.20 U/L) (P = 0.035) (Fig. 3).
205 PON-1 activity was significantly lower in cats with the effusive FIP compared to NON FIP and
206 CNTR group (P <0.0001). Nevertheless, cats with the non-effusive FIP showed significantly
207 lower PON-1 activity compared with the CNTR group (P = 0.0003), but values were not
208 different from those of the NON FIP group (P = 0.44).
209
210 *Diagnostic performance of PON-1*
211 The analysis of ROC curve highlighted a statistical difference from the no-discrimination
212 line (P <0.0001), with an AUC of 88.7% (CI 95% =0.83 – 0.94) (Fig. 4). Based on the ROC
213 curve, the threshold that maximizes the diagnostic power of the test, providing equal sensitivity
214 and specificity, corresponds to a PON-1 activity of 51.40 U/L (Se and Sp = 82%).
215
216 **Discussion**

PON-1 acts as a negative acute phase reactant in cats, similarly to other species (Giordano et al., 2013; Rossi et al., 2013; Rossi et al., 2020). From this perspective, the lower PON-1 activity observed in the FIP group compared to the other groups was not surprising, especially given the strong inflammatory response and the oxidative stress that characterize FIP (Regan et al., 2009; Tecles et al., 2015). Pro-inflammatory cytokines such as IL-6, TNF-α and IL-1β, (Montorfano et al., 2014) enhance the oxidative stress via macrophages and neutrophils respiratory burst activity that ends in the production of reactive oxygen species (ROS) (Kumar et al., 2010). Indeed, during FIP, pro-inflammatory cytokines increase the release of the bone marrow neutrophils reservoir and decrease their apoptotic rate (Paltrinieri et al., 2008; Takano et al., 2009; Paltrinieri et al., 2020). The massive recruitment of the neutrophilic component, in turn, enhances the oxidative stress through the release of strong oxidant components. A previous study had highlighted how PON-1 activity decreases in cats affected by infectious diseases including FIP (Tecles et al., 2015). This may likely occur due to its dual role as negative acute phase reactant and antioxidant compound, as demonstrated in other species (Rossi et al., 2014; Ruggerone et al., 2020). Indeed, PON-1 tends to decrease significantly in those inflammatory processes that involve a strong oxidative stress. So, a lower PON-1 activity value was expected in FIP affected cats, as observed in the present study.

In the NON FIP group, PON-1 activity resulted significantly higher than in FIP cats, but lower than in CNTR cats. This result may depend on the high number of cats with neoplasia, since one anti-tumoral mechanism is the production of ROS by macrophages (Kumar et al., 2010). Additionally, in some neoplasia (e.g. renal carcinoma), the neoplastic cells themselves produce ROS, to enhance their survival (Toyokuni et al., 1995). However, it is unlikely that neoplasia could cause the same systemic effect of a strong inflammation as FIP. The lowest values of PON-1 activity in the NON FIP group were recorded in cats with septic effusions.

Sepsis determine a huge inflammatory response, often associated with massive oxidative damage, thus possibly inducing a PON-1 decrease (Bojic et al., 2014).

A possible limitation of the study is that FIP was IHC only in 19/34 cats of the FIP group. Nevertheless, FIP was the most likely diagnosis in all the cats of this group, based on clinical and laboratory findings. Moreover, PON-1 activity did not significantly differ in cats with confirmed or presumptive FIP and, in both subgroups, it was lower than that of NON FIP and CNTR cats. This result further supports FIP in those cats in which IHC was not performed.

In the effusive form, PON-1 activity was decreased compared to the non-effusive form. This result is consistent with the higher inflammatory component of the effusive form. Indeed, in the non-effusive form, a partially protective cellular mediated immune response causes the destruction of infected monocytes and, in turn, a lower viral spread (Pedersen, 2014a). For this reason, usually granulomas are more localized. Conversely, in the effusive form, pyogranulomas are more diffused and an antibody-dependent enhancement (ADE) mechanism seem to promote inflammation (Paltrinieri et al., 2020). Since neutrophils and macrophages are the ROS major producer, it is possible to conclude that, in the effusive form, a strong inflammation together with marked oxidative stress lead to an evident decrease in PON-1 activity. The localization and amount of the granulomas could also have influenced the results. As an example, one cat showed a PON-1 value that falls within the reference interval. In this case, few IHC positive lesions were found in the cerebellum only. The strict localization and the presence of the blood-brain barrier could explain a decreased strength and efficacy of the immune response.

Finally, the analysis of the ROC curve demonstrated that PON-1 may differentiate cats

with FIP from cats without FIP but with similar clinical presentation. In a previous study, performed with a different substrate, PON-1 was decreased in FIP cats compared to healthy cats but no differences were observed with other inflammatory conditions (Tecles et al., 2015). Based on sensitivity and specificity, cats with PON-1 values lower than 51.40 U/L have about 5 folds (LR+ = 4.65) probability to have FIP. However, this threshold is not clinically relevant, because it includes several false positive and negative results. Given the emotional impact of FIP diagnosis on cats' owners, it is relevant to minimize false positive results by increasing the test specificity. A cut-off value of PON-1 equal to 24.90 U/L allows to maximize the specificity (LR+ = 18.38; Sp = 97.6%; Se = 44.1%). Indeed, a cat with a PON-1 value lower than this threshold had 18 folds the probability to be FIP affected. In this study, only 3/54 cats of the NON FIP group had PON-1 values lower than this threshold. These cats had a septic effusion, thus is not surprising to find a low value of PON-1 activity. However, in these cases, bacteria microscopical observation in the effusion easily allows to rule out FIP. This cut-off could be thus suggested when a confirmation of FIP is needed, considering that *in vivo* diagnosis is sometimes challenging. On the contrary, when false negative results could impact the health management of multicats environments (i.e. catteries) interfering with the identification of FIP cats, a cut-off that maximize the sensitivity should be preferred. In this case, FIP should be ruled out only in cats with PON-1 values higher than 78.30 U/L (LR- = 0; Se = 100%; Sp = 50%). This value falls inside the reference interval for PON-1 activity in the healthy population (57.8 – 157.3 U/L; Rossi et al., 2020).

As already reported, the result of a single test is not sufficient to confirm FIP *in vivo* (Tasker, 2018), especially without effusions. Results of this study show how PON-1 measurement is not an exception; nevertheless, very low PON-1 values could support the suspicion of FIP. PON-1 measurement through a paraoxon based method is very easy to

perform and could be set out in every laboratory. Moreover, spectrophotometric evaluation provides quicker results than the AGP immune radial diffusion. With respect of other methods used for the measurement of PON-1, the paraoxon-based method gives results on a wider numerical scale, allowing relevant clinical evaluations.

Conclusions

This study highlighted that PON-1 activity is a possible biomarker of FIP. Although the good diagnostic performances obtained in this study, the association with other laboratory tests, especially in dubious cases, is advisable. It could be interesting, in the future, to better investigate the role on PON-1 in non-effusive FIP that were here underrepresented. Recent developments in treatments against FIP (Addie et al., 2020; Dickinson et al., 2020) could also add new perspectives on the role of PON-1 as a biomarker to monitor treatment efficacy.

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Conflict of interest statement

None of the authors has any financial or personal interest that could influence or bias the content of the paper.

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452

Table 1

Breed, gender and median age of cats enrolled in the three groups. The median age is expressed in years.

Group	Breed	Gender	Median Age (Min - Max)
CNTR (*n* =71)	DSH (*n* = 32), Ragdoll (*n* = 27), Maine Coon (*n* = 3), Sphynx (*n* = 3), British Shorthair (*n* = 1), Persian (*n* = 1), Siberian (n = 1), NR (*n* = 3)	F (*n* = 26), M (*n* = 43), NR (*n* = 3)	2 y (4 mo – 13 y)
NON FIP (*n* = 54)	DSH (*n* = 36), Maine Coon (*n* = 3), Ragdoll (*n* = 3), Bengal (*n* = 1), British Shorthair (*n* = 1), Exotic Shorthair (*n* = 1), Russian blue (*n* = 1), Sphynx (*n* = 1), NR (*n* = 8)	F (*n* = 21), M (*n* = 28), NR (*n* = 5)	8.5 y (4 mo – 19 y)
FIP (*n* = 34)	DSH (*n* = 23), British Shorthair (*n* = 2), Siberian (*n* = 2), Bengal (*n* = 1), Exotic Shorthair (*n* = 1), Persian (*n* = 1), Scottish fold (*n* = 1), Sphynx (*n* = 1), NR (*n* = 3)	F (*n* = 12), M (*n* = 19), NR (*n* = 4)	1 y (4 mo – 10 y)

DSH: domestic shorthair; F: female; M: male; mo: months; NR: not reported; y: years

458 **Table 2**

459 Laboratory results for cats with highly suspected FIP (PRESUMPTIVE FIP subgroup). All
460 the cats had a cavitary effusion and severe clinical conditions that always ended in
461 spontaneous death or euthanasia (Stranieri et al., 2018).

ID	LY (<1.5 *10^6/L)	TP (>80 g/L)	ALB (<21 g/L)	A:G (<0.8)	SPE	AGP (>1.5 mg/mL)	PCR	ΔTNC (>2.5)	CYTO	OTHER
126	X		X	X	X	X	X	X	X	J
127	X	X	X	X	X	X		X	X	
128	X		X	X	X			X	X	ICC[b]
129	X		X	X	X			X		
130		X	X	X	X	X		X	X	J, N
131		X	X	X			X	X	X	
132		X	X	X				X	X	J
134		X	X	X	X		X[a]		X	
135			X	X	X		X	X	X	N
136			X	X			X	X	X	N
137			X	X	X			X	X	N
138	X		X	X				X	X	
139	X		X	X	X			X	X	J, N

140	X		X	X	X			X	X
141	X	X	X	X	X				

LY: absolute lymphocyte count measured on peripheral blood; TP: serum total protein; ALB: serum albumin; A:G: albumin to globulin ratio; SPE: serum protein electrophoresis; AGP: serum alpha-1-acid glycoprotein; CYTO: effusion cytology, J: jaundice; N: necropsy.

[a] RT-PCR targeting the 3' UTR region was always performed on effusion except in this case, in which it was performed on cerebrospinal fluid

[b] Immunocytochemistry was performed of effusion

Figure legends

Fig. 1. Paraoxonase (PON-1) activity (U/L) recorded in FIP, NON FIP and CNTR cats. The boxes indicate the I–III interquartile range (IQR), the horizontal line indicates the median values, whiskers extend to further observation within the I quartile minus 1.5*IQR or to further observation within the III quartile plus 1.5*IQR. The shaded area reported the reference interval of PON-1 for healthy cats (Rossi et al., 2020).

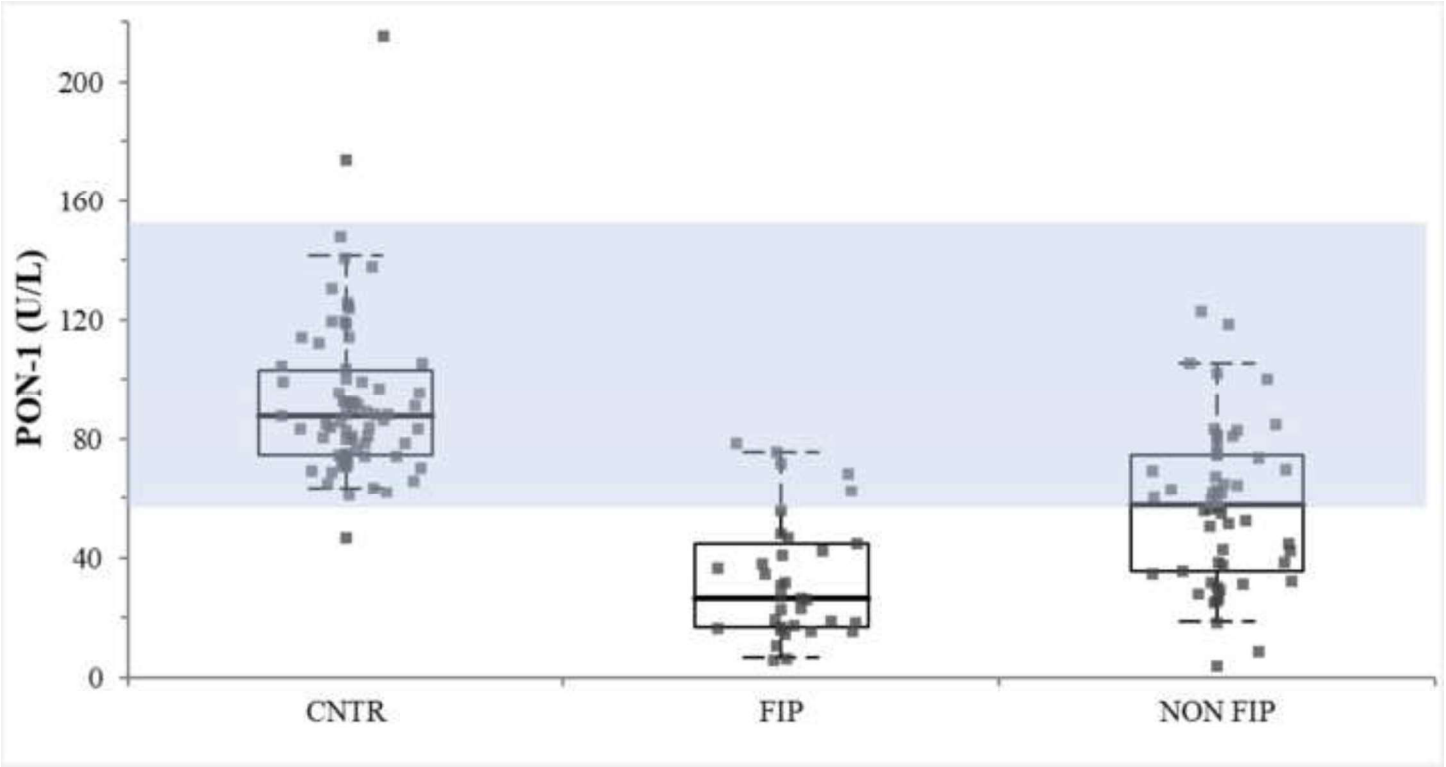
Fig. 2. Results obtained from the comparison of the value of PON-1 (paraoxonase 1) activity (U/L) among the subgroup of CONFIRMED, PRESUMPTIVE FIP, and groups NON FIP and CNTR. The boxes indicate the I–III interquartile range (IQR), the horizontal line indicates the median values, whiskers extend to further observation within the I quartile minus 1.5*IQR or to further observation within the III quartile plus 1.5*IQR. The shaded area reported the reference interval of PON-1 for healthy cats (Rossi et al., 2020).

Fig. 3. Results obtained from the comparison of the value of PON-1 (paraoxonase 1) activity (U/L) among the subgroup of DRY FIP, WET FIP and the groups NON FIP and CNTR. The boxes indicate the I–III interquartile range (IQR), the horizontal line indicates the median values, whiskers extend to further observation within the I quartile minus 1.5*IQR or to further observation within the III quartile plus 1.5*IQR. The shaded area reported the reference interval of PON-1 for healthy cats (Rossi et al., 2020).

Fig. 4. Receiver Operating Characteristic (ROC) curve built using the values of PON-1 (paraoxonase 1) activity (U/L) for the diagnosis of FIP. The central line represents the no-discrimination line.

Figure 1

Click here to access/download;Figure;Fig.1.tif



Livello incollato

Figure 2

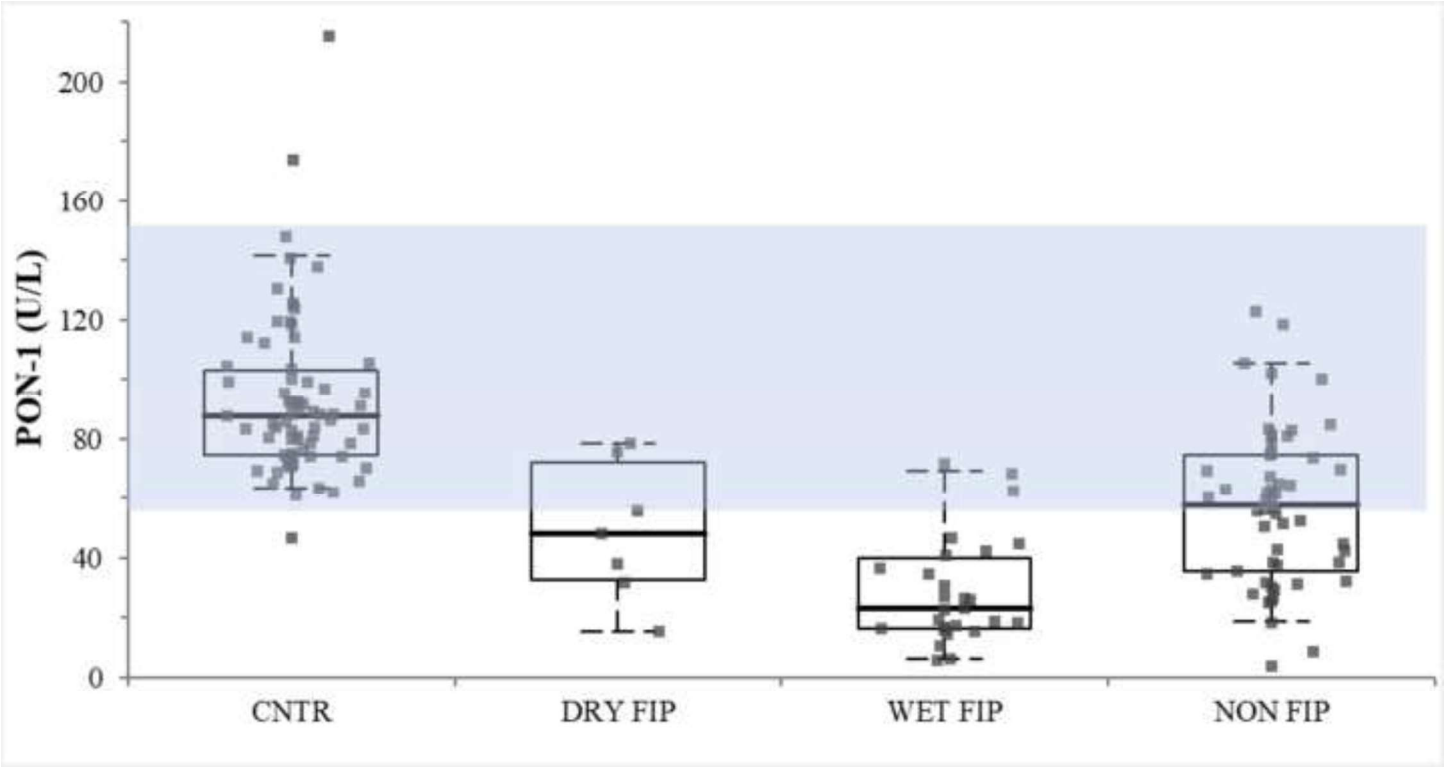


Figure 3

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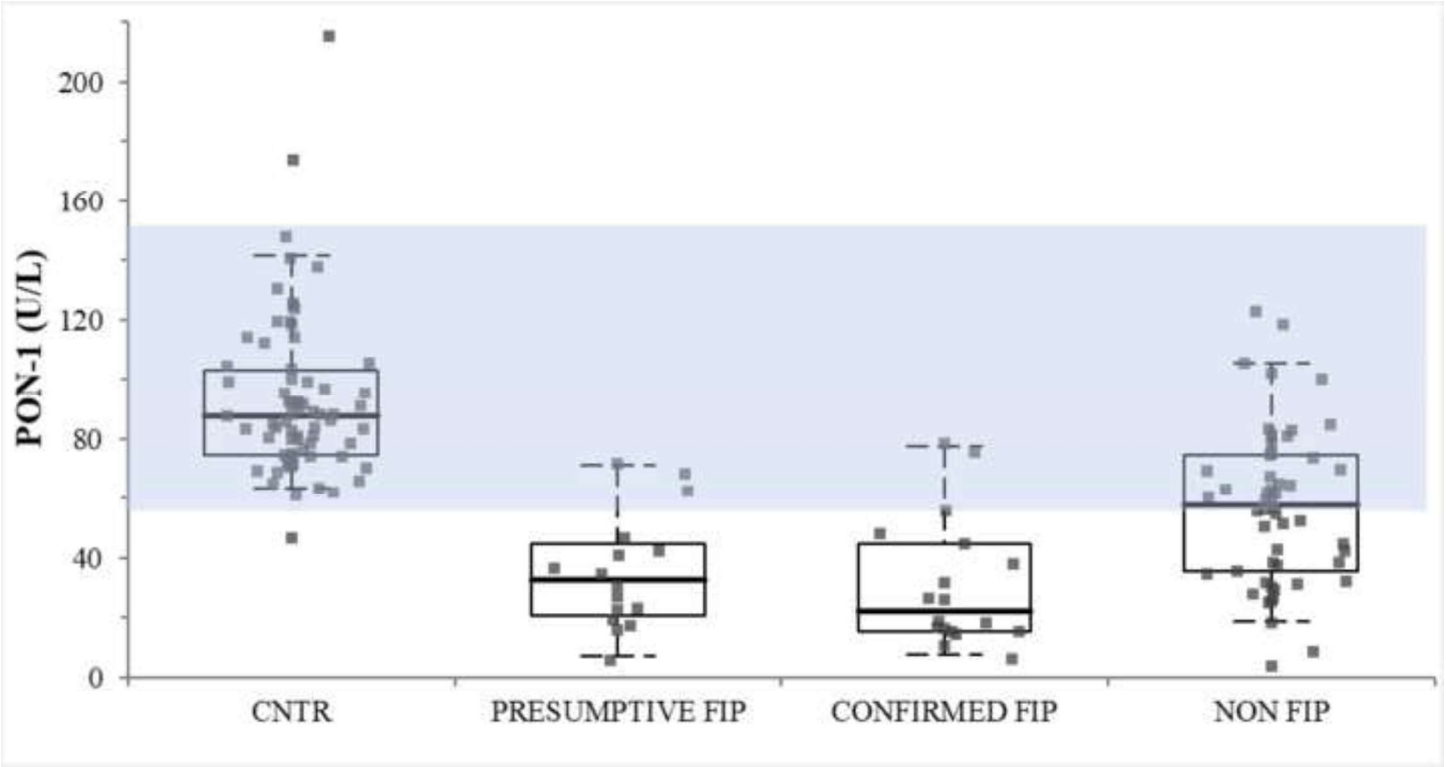

Figure 4

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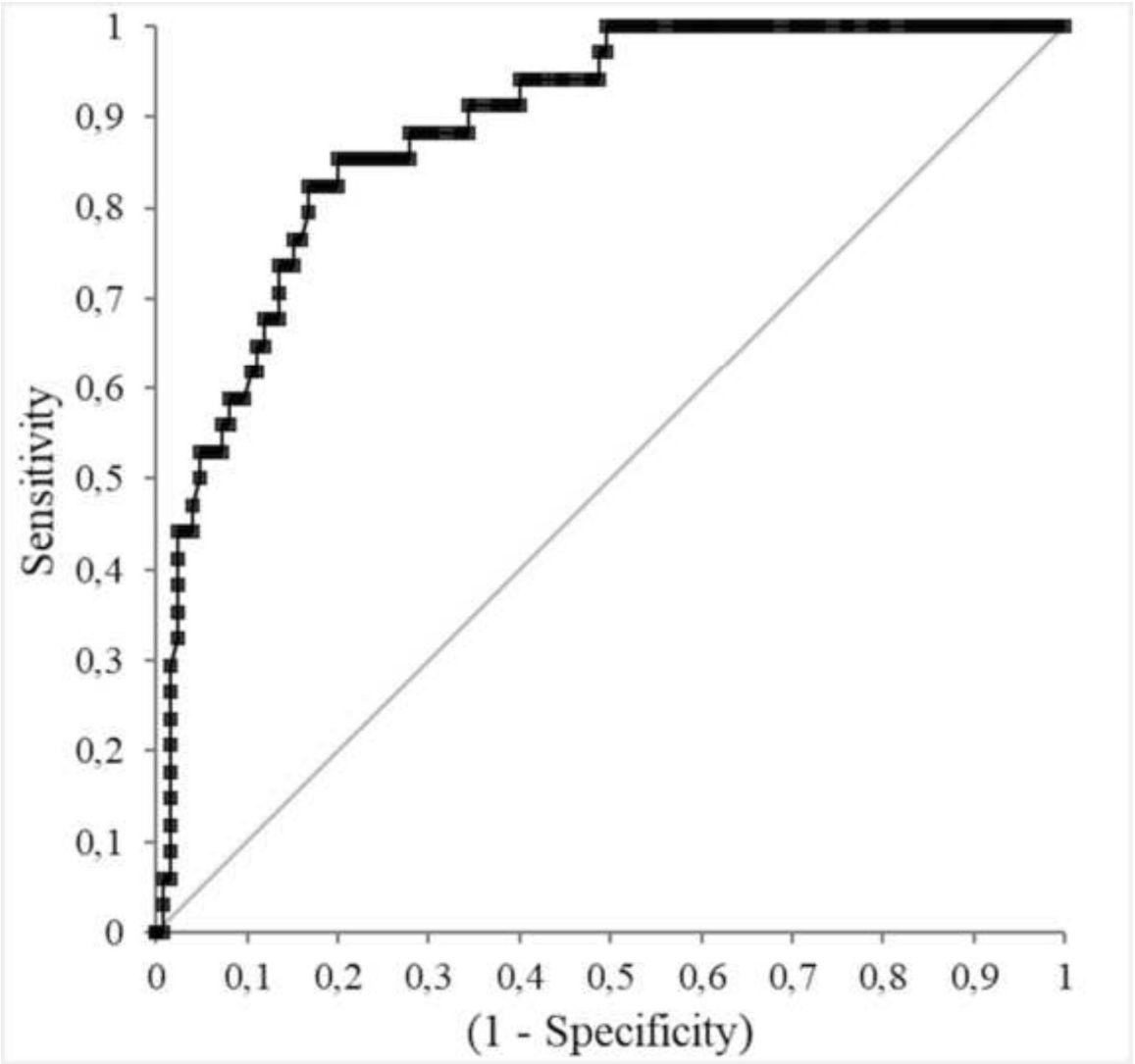

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Highlights

- Feline infectious peritonitis is associated with strong inflammation and oxidation
- Paraoxonase-1 (PON-1) is an antioxidant and a negative acute phase reactant
- PON-1 activity lowest values were observed in cats with FIP
- PON-1 seems accurate in discriminating FIP from other similar clinical conditions
- Measurement of PON-1 activity could serve as a possible biomarker of FIP

Dear Editor,

the manuscript here enclosed and titled ‘Role of paraoxonase-1 (PON-1) as a diagnostic marker for feline infectious peritonitis (FIP)’ is submitted to be considered for publication on *The Veterinary Journal*

The manuscript has been reviewed by an English-speaking scientist and a thorough format editing has been performed before submission in order to comply with journal guidelines.

I would certify that the manuscript has not be submitted to other Journals. Preliminary results of this study were presented at the XVIIIth ISACP (International Society for Animal Clinical Pathology) annual congress in Tokyo, 4-8th August, 2018.

Sincerely,
Angelica Stranieri