

Pathology and behavior in feline medicine: investigating the link between vomeronasalitis and aggression.

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Keywords:	aggression, behavior, cat, inflammation, pathology, pheromones, vomeronasal organ
Abstract:	<p>Objectives The aim of this study was to investigate if the feline vomeronasal organ (VNO) can be affected by inflammatory lesions and if these changes could be associated to behavioral alterations.</p> <p>Methods VNOs from 20 cats were sampled during necropsy, submitted to routine tissue processing and stained in hematoxylin and eosin for histopathological evaluations. Of the 20 cats, data about the presence of aggressive behaviors towards cats or humans at the moment of the death were collected. Inflammatory lesions were classified depending on the duration of the process in acute or chronic inflammation both in vomeronasal sensory epithelium (VNSE) and in non-sensory epithelium (NSE). The Fischer exact test was used to compare VNO inflammation to behavioral data.</p> <p>Results The VNSE was inflamed in 11/20 VNOs (55%) while the NSE in 13/20 (65%). Overall, the VNO was affected by inflammation in 14/20 (70%) cats, and all the lesions were classified as chronic processes. The cats presenting intraspecific aggressive behaviors were 5/20 (25%), while those presenting aggression against humans were 8/20 (40%). The Fisher exact test showed a statistical significant correlation between the inflammation of the VNSE and the intraspecific aggression ($P=0.038$). No statistical correlations were observed between VNSE inflammation and aggression towards humans and between NSE inflammation and aggression towards</p>

	<p>cats or humans.</p> <p>Conclusions and relevance Our results show for the first time the existence of vomeronasalitis in animals and its possible association to intraspecific aggressive behaviors. The inflammatory microenvironment could impair VNSE functionality causing intraspecific communication alterations, probably throughout a reduction of chemical communication action and perception. Due to the pivotal role of VNO in social life of cats and other species, this report provides a rationale to further investigate this disease in relation to several behavioral alterations.</p>

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Apt, the 21st of July 2015

Dear Andy Sparkes

Editor, *Journal of Feline Medicine and Surgery*

Thank you very much for your letter dated 9th July 2015. Attached herewith please find the revised copy of the manuscript **“Pathology and behavior in feline medicine: investigating the link between vomeronasalitis and aggression.”** by P. Asproni et al.

The Authors thank the Editor and the Reviewers for the thorough revision that has certainly improved the quality of our manuscript.

During the preparation of the revised manuscript we tried to follow the suggestions of both the reviewers, in order to take into account all their important opinions.

All the changes we made have been explained in the following pages.

Thanking you for your kind consideration, I take the opportunity to send my best regards.

Yours Sincerely,

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Answers to reviewers' comments

Reviewer: 1

Reviewers report for the author

Thank you for producing data on a quite novel concept in the minds of most investigators in veterinary medicine. I think with a few change you will have manuscript well worth publishing.

Title: Consider changing to "Pathology and behavior in feline medicine: Investigating the link between vomeronasalitis and aggression"

We followed reviewer's suggestion and we changed the title as he proposed.

Line 73: The last two sentences of this paragraph are confusion. I think something may be lost in translation.

We tried to rephrase the paragraph in order to clarify its meaning.

Line 87: You mention that there is very little literature on VNO pathologies, but are you able to describe ANY sense of the prevalence of vomeronasalitis in cats? Could you offer any description of the clinical signs which might suggest this pathology to the practitioner?

Unfortunately, up to now we are not able to recognize some clinical signs of feline vomeronasalitis in living cats. We thought that the internal position of this organ and the fact that it is enclosed between oral and nasal cavities make the finding of these signs very difficult. One of the next steps of our project is to find a way to diagnose this condition in living animals by means of diagnostic tools other than histology, to give to the practitioner an important help during the clinical examination.

Line 96: When you say that 14 cats died, does that mean they died naturally of other underlying causes or that they were euth? Did the cats that were put to sleep for behavioral reasons have any PHYSICAL clinical signs at the time of death?

Of the 20 cats, 6 were euthanized due to the presence of untreatable aggressive behaviors, against other cats, humans or both. These animals presented only these behavioral signs and they did not present any other clinical signs. The 14 cats that died for other causes (not euthanasia) were animals submitted to necropsy by the practitioner to assess the cause of death as routine. These animals presented clinical signs linked to their conditions. However, none of these signs of all the 20 cats can be, on our opinion, linkable to the vomeronasalitis, as we specify before in this letter.

Line 105: Why check for acute or chronic? Did you anticipate the results to be different?

We selected a classification commonly used in animal pathology. Considering VNO position and its morphological similarity with nasal epithelia, we also supposed a similar condition between these two structures. As cited in our manuscript, feline nasal inflammation are commonly classified in acute and chronic (Henderson et al, 2004; Reed, 2014).

Results: Do you have information on the households of each cat? Did all cats live in multicat households? This is important to know if you are measuring for aggressio between cats in a household. You found a link between inter-cat aggression and VNSE, but could there have been fewer cats reported as having intercat aggression simply because they lived in a single cat



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household? I wonder if you might have had a higher report of intercat problems if all cats in the study were in multicat homes?

All the 20 cats included in this study lived with other cats (range: 2 to 5 cats for home).

Results: Did you obtain information about the aggression so that you could determine if the aggression was fear based? Conflict based? or Redirected? Just curious if motivation for aggression could be inferred from the questionnaire or not.

We do not have these information. Another aspect that we would investigate in the future is to distinguish the different types of aggression in relationship with vomeronasalitis, as we specified in the end of the discussion.

Reviewer: 2

Reviewers report for the author

I would like to apologize for the delay in the analysis of the paper JFMS-15-0088 entitled "Pathology and behavior in feline medicine: first description of feline vomeronasalitis and its relationship with aggressive behaviors". I truly believe the topic is unique and raises important concerns regarding the link between disease and behaviour which is so relevant in feline medicine. Vomeronasalitis in cats and its possible association with feline behaviour has never been reported before and so I think the paper is worth publishing.

However, I think the paper should be re-write more carefully as we don't have evidence to say vomeronasalitis is a factor in the onset of cat-cat aggression. Maybe cat-cat aggression as well as vomeronasalitis (as such common entities) may be a consequence of a third environmental part that it is not identified at this point. We surely need a bigger sample size to be more confident that the association was not just coincidental. Thus, the paper should be more focused on the high incidence of vomeronasalitis in cats which per se is very interesting! Thus, on a second level describe the "possible association" found. Another point is that it should be emphasized that the association found was between vomeronasalitis and cat-cat aggression perceived by the owners.

We tried to rephrase some paragraphs in order to explain that the presence of a statistical significant association is not a sufficient evidence to talk about a "cause-effect relationship". It is not our aim to present vomeronasalitis as the main cause of intraspecific aggression, our purpose is to introduce this condition as a possible cofactor of a very complex disorder as the cat-cat aggression is. We hope that the changes that we performed throughout the discussion help to reach this purpose.

Other minor corrections:

- I prefer the term "disease" instead of "pathology"

We changed "pathology" into "disease" where appropriate.

- Line 21 – take "animal" out from the expression. "animal behavioral alterations"

We followed reviewer's suggestion.

- Line 25 – pay attention on words that are joined such as "catswere". This happens several other times along the whole paper. Please correct



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In the Word file these words are not joined. We also observed this problem in the PDF, maybe it depends on the Word/PDF conversion system.

- Line 46 – use “could” rather than “seem” as there is not any evidence from the paper of the mechanisms behind the association between vomeronasalitis and cat-cat aggression
We changed “seems to” into “could”.
- Line 63 “it passes to the oral cavity...” gives the impression that the organ extends into the oral cavity which is not correct. Please correct the phrase
We tried to clarify this sentence.
- Linha 71 “considered” instead of “consider”
We changed “consider” into “considered”.
- Line 78 “chemical chomunication sent thanks to marking behavior” does not sound right
We rephrased this sentence.
- Line 81 “and organize their social life in general” sounds better
We followed reviewer suggestion.
- Line 81 “The inability to understand feline communication and the alterations of someone of this kind of marking behavior could be the sign of a behavioral modification like aggressiveness related to a perturbation of the social and spatial environment” does not sound right. Please, correct the phrase
We tried to simplify this sentence.
- Line 90 “can be associated with...” rather than “can be a fator in the onset...”
We followed reviewer’s suggestion.
- Line 165 “seems to be a fator in the onset” is not appropriate as you did not study cause and effect. You simply found an association that needs to be further investigated
We rephrased in order to follow reviewer’s suggestion.
- Line 214 “behavior modification” refers to a technique of promoting behaviour change withinh behaviour therapy. Thus, when talking about aggression do prefer “behavior alteration”
We rephrased in order to clarify this aspect.

18 **Abstract**

19 *Objectives*

20 The aim of this study was to investigate if the feline vomeronasal organ (VNO) can be
21 affected by inflammatory lesions and if these changes could be associated to behavioral
22 alterations.

23

24 *Methods*

25 VNOs from 20 cats were sampled during necropsy, submitted to routine tissue
26 processing and stained in hematoxylin and eosin for histopathological evaluations. Of
27 the 20 cats, data about the presence of aggressive behaviors towards cats or humans at
28 the moment of the death were collected. Inflammatory lesions were classified depending
29 on the duration of the process in acute or chronic inflammation both in vomeronasal
30 sensory epithelium (VNSE) and in non-sensory epithelium (NSE). The Fischer exact
31 test was used to compare VNO inflammation to behavioral data.

32

33 *Results*

34 The VNSE was inflamed in 11/20 VNOs (55%) while the NSE in 13/20 (65%). Overall,
35 the VNO was affected by inflammation in 14/20 (70%) cats, and all the lesions were

36 classified as chronic processes. The cats presenting intraspecific aggressive behaviors
37 were 5/20 (25%), while those presenting aggression against humans were 8/20 (40%).
38 The Fisher exact test showed a statistical significant correlation between the
39 inflammation of the VNSE and the intraspecific aggression ($P=0.038$). No statistical
40 correlations were observed between VNSE inflammation and aggression towards
41 humans and between NSE inflammation and aggression towards cats or humans.

42

43 *Conclusions and relevance*

44 Our results show for the first time the existence of vomeronasalitis in animals and its
45 possible association to intraspecific aggressive behaviors. The inflammatory
46 microenvironment could impair VNSE functionality causing intraspecific
47 communication alterations, probably throughout a reduction of chemical
48 communication action and perception. Due to the pivotal role of VNO in social life of
49 cats and other species, this report provides a rationale to further investigate this disease
50 in relation to several behavioral alterations.

51

52

53

54 **Introduction**

55 The vomeronasal organ (VNO) is a peripheral chemosensory structure involved in the
56 detection of pheromones in vertebrates.¹ In cats, this organ has been widely studied, and
57 its key-role in feline behavior and communication has been clearly demonstrated.^{2,3}
58 Salazar and colleagues showed that the anatomical and the histological morphology of
59 the feline VNO do not differ from the other species.⁴ In fact, the feline VNO is
60 surrounded by the vomeronasal cartilage and it is located in the nasal cavity in contact
61 with the vomer, the palatine process of the palatine bone and the incisive bone.⁴
62 Laterally, it is in contact with the nasal mucosa, and ventrally it opens into the oral
63 cavity through the palatine fissure.⁴ From a histological point of view, VNO lumen is
64 delimited by two types of epithelia: the non-sensory epithelium (NSE) and the
65 vomeronasal sensory epithelium (VNSE).⁴ The NSE closely resembles the respiratory
66 epithelium of the nasal cavity,^{4,5} while the VNSE presents several similarities with the
67 sensorial epithelium of the main olfactory system.^{4,5}

68 Up to now, evidences of VNO alteration have been rarely reported in literature.
69 In 1974, Loo and Chin described the presence of lymphatic nodules in the VNO of tree
70 shrews, but this finding was not considered a pathological change.⁶ More recently,
71 microabscesses were observed in the VNO of rabbits after the instillation of
72 experimental vehicles in the nasal cavity.⁷ No reports described instead spontaneous
73 pathological changes of the VNO in other animals. In fact, only induced VNO

74 alterations have been reported, by several studies finding that the surgical removal of
75 the VNO leads to the marked increase of deficits in social and reproductive behavior in
76 various species,^{8,9,10}

77 Pheromones are involved in territorial marking behavior in cats and in complex
78 social exchanges.¹¹ Chemical messages emitted by means of marking behaviors are a
79 more permanent form of communication than either postures or vocalizations.¹²
80 Individuals can space chemical messages to prevent meetings, recognize territory,
81 control reproduction and organize their social life in general.¹² Alterations in marking
82 behaviors and impairments in chemical communication understanding could lead to
83 aggression related to perturbations of the social and spatial environment.¹² The VNO is
84 the organ that receives chemical messages and is the first step for the integration of a
85 chemical message and at the base of the induction of a specific reactions.¹³

86 Considering the absence of substantial literature about VNO diseases and the
87 pivotal role of the VNO in the social life and communication of the cat, the aim of this
88 study was to evaluate if this organ can be affected by inflammatory alterations, and if
89 these conditions can be associated with feline aggressive behaviors.

90

91 **Materials and Methods**

92 Twenty cats (10 males and 10 females) aged between 1 and 13 years (mean = 7.8 years
93 \pm 3.9 years) were included in this study. Six subjects were humanly euthanized
94 (intravenous injection of 0.7 ml/kg of pentobarbital sodium) due to the persistence of
95 severe aggressive behaviors,¹⁴ while 14 died for different organic causes. All the cats
96 were necropsied to exclude the presence of organic pathologies that could influence
97 their behavior. During the necropsy, the VNOs of all the cats were sampled, fixed in
98 10% buffered formalin (pH 7.4) for 48 hours and processed by routine methods for
99 histological analyses. VNOs were cut transversally respect their length in order to
100 observe both the NSE and the VNSE at the histological exam. Four- μ m-thick sections
101 were stained with haematoxylin and eosin for the histopathological analysis. Due to the
102 different roles played by the NSE and the VNSE side of the VNO,^{4,5} they were
103 considered as separated entities for the histopathological evaluation and the statistical
104 analysis. Inflammations were classified as acute or chronic, depending on the type of
105 cellular infiltrate and the duration of the process.^{15,16}

106 About all the subjects, data regarding the presence of aggressive
107 behaviors against humans or against the other cats were collected by means of a
108 questionnaire completed by the owners at the moment of the death.

109 Statistical analysis was performed using 9.4 SAS software (SAS Institute Inc.,
110 Cary, NC, USA). The Fisher exact test was used to investigate the significance of the
111 relationship between VNSE and NSE inflammation and the following parameters: sex,

112 age, presence of intraspecific aggression and presence of aggression against humans,
113 using the FREQ procedure. Statistical significance was based on a 5% (0.05)
114 significance level.

115

116 **Results**

117 *Histological analysis*

118 The VNSE was affected by inflammation in 11 of the 20 samples (55%) included in this
119 study, while 13/20 (65%) were affected by inflammation of the NSE. Overall, the VNO
120 was inflamed in 14/20 (70%) cats, and 10 of the 20 VNOs (50%) presented a concurrent
121 inflammation of the two epithelia. Of the 4 discordant cases, 3 were affected only in the
122 NSE and 1 only in the VNSE. Six of the 20 VNOs (30%) were not altered. Data
123 regarding VNO inflammation are illustrated in Table 1.

124 All the 14 vomeronasalitis were classified as chronic inflammations. Nine of
125 these lesions were also subclassified as follows: 6/20 (30%) were chronic-active
126 processes, 2/20 (10%) were pyogranulomatous inflammations, and 1/20 (5%) was a
127 VNO vasculitis. This latter affected the VNO of a cat dead for an effusive form of
128 Feline Infectious Peritonitis (FIP), and it was characterized by the infiltration of
129 inflammatory cells around blood vessels and vascular congestion. In the 5/20 (25%)
130 VNOs affected by a classic chronic process, the inflammatory infiltrate was mainly

131 composed by small lymphocytes, with a minor involvement of plasma cells and
132 macrophages (Figure 1A and 1B). The 6 chronic-active vomeronasalitis were
133 characterized by the same cellular infiltrate of the chronic ones, but with the concurrent
134 involvement of mature neutrophils, sign of the restarting of the inflammatory process.
135 In the two VNOs presenting pyogranulomatous inflammation, the cellular infiltrate was
136 composed by macrophages and neutrophils, often affected by degenerative processes
137 (Figure 1C). In chronic, chronic-active and pyogranulomatous lesions, inflammatory
138 cells were mainly infiltrated in the VNO soft tissue under the NSE and VNSE and
139 among the epithelial layers (Figure 1). In 4 VNOs, inflammatory cells arrived to mildly
140 infiltrate vomeronasal glands and nerves. The necrosis of the VNO epithelia was rarely
141 observed and commonly associated to the detachment of the necrotic cells into the
142 lumen (Figure 1B and 1D). Furthermore, the inflammation of VNSE and NSE was often
143 accompanied by the hydropic degeneration of the epithelial cells (Figure 1C).

144

145 *Behavioral data*

146 Of the 20 cats, 5 (25%) presented aggressive behaviors against the other cats and 8
147 (40%) against humans. Two of the 20 cats (10%) presented aggression both towards
148 cats and humans, while 9/20 subjects (45%) were not aggressive at all. Data regarding
149 aggressive behaviors are reported in Table 2.

150

151 *Statistical analysis*

152 The Fisher's exact test showed a statistically significant correlation between VNSE
153 inflammation and the presence of aggressive behaviors against cats ($P = 0.038$). No
154 correlation was observed between VNSE inflammation and sex, age and aggression
155 against humans ($P > 0.05$). However, the test showed a statistical trend between VNSE
156 inflammation and female cats ($P = 0.065$). No statistical significant correlation or trend
157 were instead observed between NSE inflammation and the other parameters included in
158 the statistical analysis.

159

160 **Discussion**

161 This study describes for the first time the feline vomeronasalitis and its possible
162 association to aggressiveness in cats. Our data showed that a large percentage of feline
163 VNOs can be affected by chronic inflammation, which can involve both the NSE and
164 VNSE. The statistical analysis showed that only the VNSE inflammation could be
165 associated to intercat aggression perceived by the owners.

166 The importance of the VNO in several aspects of animal behavior has been
167 widely demonstrated by the surgical removal of this structure.⁸ In fact, after VNO

168 ablation or making VNO inaccessible, animals showed marked deficits in social and
169 reproductive behavior.^{9,10,17,18,19} On the other hand, there are no studies in literature
170 focused on VNO diseases and VNO alterations have been rarely reported only as a
171 secondary finding in experimental condition.^{6,7} The presence of lymphatic nodules
172 under the vomeronasal epithelium has been described in tree shrews,⁶ while in rabbits it
173 has been observed the formation of VNO microabscesses as consequence of
174 experimental vehicle instillation in the nasal cavity.⁷

175 Due to the absence of bibliography about vomeronasalitis, we can draw a
176 parallel between our findings and feline chronic rhinitis, also considering the similar
177 position and histomorphology of the two structures.⁴ Chronic rhinitis is a common
178 finding in cats of any age, and it is mainly composed by a lymphoplasmacytic
179 infiltrate.^{15,16} A number of different causes can be responsible for the onset of this
180 condition, but the viral etiology is the more involved in this process.¹⁶ Bacterial
181 infections are also commonly identified during feline chronic rhinitis, but this finding is
182 normally considered a secondary consequence of the viral infection.¹⁶ The similar
183 anatomical position suggests that the feline chronic vomeronasalitis may be caused by
184 the same etiologies of the chronic rhinitis. A peculiar data of our study is that the feline
185 VNO was affected just by chronic processes, while neutrophils were involved only in
186 association to a pre-existing lymphocytic or macrophagic infiltrate. In feline rhinitis,
187 neutrophilic infiltrate is considered twice as common as the lymphocytic type, and it is

188 associated to acute and suppurative processes.¹⁶ In our study, we did not observe acute
189 suppurative vomeronasalitis, and this finding could maybe suggest some differences in
190 the immunity function between nasal and vomeronasal epithelium or in the entry
191 pathway of the etiological cause. In fact, the entry of the air in the VNO is regulated by
192 a pumping/suction mechanism that makes VNO inaccessible for the majority of the
193 time, differently from what happens in the nasal cavity.¹²

194 Even if a larger number of subjects should be investigated to verify the cause-
195 effect relationship between vomeronasalitis and intraspecific aggression, our data
196 showed that the inflammation of the VNSE was statistically correlated to intraspecific
197 aggression, while NSE inflammation was not correlated. As previously reported, VNSE
198 closely resembles the sensorial epithelium of the main olfactory system of the nasal
199 cavity.^{4,5} In humans, the chronic inflammation of the olfactory mucosa has been
200 associated to the reduction of odor perception, as a probable consequence of
201 inflammation mediators and cytokines effect on receptor neurons and nerves.²⁰ In fact,
202 the chronic exposure to tumor necrosis factor-alpha (TNF- α) leads to olfactory
203 dysfunction through reduction of neurons number, olfactory epithelium thickness and
204 nervous function in mice.^{21,22} TNF- α is a cytokine mainly produced by lymphocytes and
205 macrophages,²² cells that mainly composed the inflammatory infiltrate of the feline
206 VNOs analyzed in this study. Also another macrophagic and lymphocytic cytokine, the
207 interleukin 6 (IL-6), has been associated to human hyposmia.²³ Thus, it is authors'

208 opinion that the inflammatory microenvironment proposed as cause for humans and
209 mouse olfactory loss may also impair feline VNO during spontaneous vomeronasalitis.
210 On the contrary, NSE inflammation was not correlated with intraspecific aggression.
211 This data confirms that only the VNSE is responsible for pheromones detection, as
212 widely reported in literature.^{4,5,8} VNSE and NSE inflammations were not correlated to
213 aggression towards people, suggesting that probably in cat-human interactions other
214 factors (e.g. postural communication, vocalizations, owners' characteristics) play a
215 more crucial role.²⁴ On the contrary, our data suggest that chemical communication
216 plays a putative stronger role in intercat relationship than those factors involved in
217 human-cat interaction control.

218 Among feline behavioral alterations, intraspecific aggression is the most
219 common disorder.^{25,26} This condition can be fear-related, status- or conflict-related,
220 play-related, redirected or caused by the entry of a new subject into an pre-existing
221 group of cats.²⁶ However, until now, VNO diseases have been never proposed as
222 potential factor of intercat aggression. Our data seem to suggest that the inflammation
223 of this organ and its following dysfunction could lead to intraspecific communication
224 alterations causing intercat aggression. These data open a new way of investigation that
225 links pathology to behavioral medicine. Moreover, the following studies should
226 investigate the possible correlation between vomeronasalitis and the different kind of

227 aggressiveness, in order to evaluate possible dissimilarities between them or the
228 subjects involved in the social interactions (familiar or unfamiliar subjects).

229

230 **Conclusions**

231 To the best of our knowledge, this is the first study describing VNO diseases in cats or
232 in other species. Due to the pivotal role of this organ in animal behavior, this first
233 report reveals several clinical and behavioral implications of VNO pathological
234 changes. Even if a wider number of VNO samples are needed to draw firmer
235 conclusions, our data suggest that vomeronasalitis could be a cofactor in the onset of
236 intercat aggressive behavior.

237

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239 This research received no grant from any funding agency in the public, commercial or
240 not-for-profit sectors.

241

242 **Conflict of interest statement**

243 The authors declare that there is no conflict of interest.

244

245

246

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249 **specialized for detecting pheromones?** *Trends Neurosci* 2006; 29: 1-7.
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321

322 **Figure legend**

323

324 **Figure 1** Cat, vomeronasal organ. (A) Feline chronic vomeronasalitis affecting the
325 vomeronasal sensory epithelium (VNSE) and the non-sensory epithelium (NSE).
326 (haematoxylin and eosin stain, x 40). (B) Feline chronic vomeronasalitis affecting
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328 plasma cells are also present. VNSE is totally disrupted by the presence of
329 inflammatory cells and clusters of necrotic epithelial cells are observable into the VNO
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331 vomeronasalitis affecting VNSE. Macrophages and neutrophils massively infiltrate the
332 soft tissue under VNSE, which is reduced in thickness and partially necrotic.
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334 VNSE. Lymphocytes and plasma cells interspersed within nerves (arrowheads) and
335 vomeronasal glands (arrows). (haematoxylin and eosin stain, x 400).

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1 **Pathology and behavior in feline medicine: investigating the link between**
2 **vomeronasalitis and aggression.**

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18 **Abstract**

19 *Objectives*

20 The aim of this study was to investigate if the feline vomeronasal organ (VNO) can be
21 affected by inflammatory lesions and if these changes **could** be associated to behavioral
22 alterations.

23

24 *Methods*

25 VNOs from 20 cats were sampled during necropsy, submitted to routine tissue
26 processing and stained in hematoxylin and eosin for histopathological evaluations. Of
27 the 20 cats, data about the presence of aggressive behaviors towards cats or humans at
28 the moment of the death were collected. Inflammatory lesions were classified depending
29 on the duration of the process in acute or chronic inflammation both in vomeronasal
30 sensory epithelium (VNSE) and in non-sensory epithelium (NSE). The Fischer exact
31 test was used to compare VNO inflammation to behavioral data.

32

33 *Results*

34 The VNSE was inflamed in 11/20 VNOs (55%) while the NSE in 13/20 (65%). Overall,
35 the VNO was affected by inflammation in 14/20 (70%) cats, and all the lesions were

36 classified as chronic processes. The cats presenting intraspecific aggressive behaviors
37 were 5/20 (25%), while those presenting aggression against humans were 8/20 (40%).
38 The Fisher exact test showed a statistical significant correlation between the
39 inflammation of the VNSE and the intraspecific aggression (P=0.038). No statistical
40 correlations were observed between VNSE inflammation and aggression towards
41 humans and between NSE inflammation and aggression towards cats or humans.

42

43 *Conclusions and relevance*

44 Our results show for the first time the existence of vomeronasalitis in animals and its
45 possible association to intraspecific aggressive behaviors. The inflammatory
46 microenvironment could impair VNSE functionality causing intraspecific
47 communication alterations, probably throughout a reduction of chemical
48 communication action and perception. Due to the pivotal role of VNO in social life of
49 cats and other species, this report provides a rationale to further investigate this disease
50 in relation to several behavioral alterations.

51

52

53

54 **Introduction**

55 The vomeronasal organ (VNO) is a peripheral chemosensory structure involved in the
56 detection of pheromones in vertebrates.¹ In cats, this organ has been widely studied, and
57 its key-role in feline behavior and communication has been clearly demonstrated.^{2,3}
58 Salazar and colleagues showed that the anatomical and the histological morphology of
59 the feline VNO do not differ from the other species.⁴ In fact, the feline VNO is
60 surrounded by the vomeronasal cartilage and it is located in the nasal cavity in contact
61 with the vomer, the palatine process of the palatine bone and the incisive bone.⁴
62 Laterally, it is in contact with the nasal mucosa, and ventrally **it opens into** the oral
63 cavity through the palatine fissure.⁴ From a histological point of view, VNO lumen is
64 delimited by two types of epithelia: the non-sensory epithelium (NSE) and the
65 vomeronasal sensory epithelium (VNSE).⁴ The NSE closely resembles the respiratory
66 epithelium of the nasal cavity,^{4,5} while the VNSE presents several similarities with the
67 sensorial epithelium of the main olfactory system.^{4,5}

68 Up to now, evidences of VNO alteration have been rarely reported in literature.
69 In 1974, Loo and Chin described the presence of lymphatic nodules in the VNO of tree
70 shrews, but this finding was not **considered** a pathological change.⁶ More recently,
71 microabscesses were observed in the VNO of rabbits after the instillation of
72 experimental vehicles **in the nasal cavity**.⁷ No reports described instead spontaneous
73 pathological changes of the VNO in other animals. **In fact, only induced VNO**

74 alterations have been reported, by several studies finding that the surgical removal of
75 the VNO leads to the marked increase of deficits in social and reproductive behavior in
76 various species,^{8,9,10}

77 Pheromones are involved in territorial marking behavior in cats and in complex
78 social exchanges.¹¹ Chemical messages emitted by means of marking behaviors are a
79 more permanent form of communication than either postures or vocalizations.¹²
80 Individuals can space chemical messages to prevent meetings, recognize territory,
81 control reproduction and organize their social life in general.¹² Alterations in marking
82 behaviors and impairments in chemical communication understanding could lead to
83 aggression related to perturbations of the social and spatial environment.¹² The VNO is
84 the organ that receives chemical messages and is the first step for the integration of a
85 chemical message and at the base of the induction of a specific reactions.¹³

86 Considering the absence of substantial literature about VNO diseases and the
87 pivotal role of the VNO in the social life and communication of the cat, the aim of this
88 study was to evaluate if this organ can be affected by inflammatory alterations, and if
89 these conditions can be associated with feline aggressive behaviors.

90

91 **Materials and Methods**

92 Twenty cats (10 males and 10 females) aged between 1 and 13 years (mean = 7.8 years
93 \pm 3.9 years) were included in this study. Six subjects were humanly euthanized
94 (intravenous injection of 0.7 ml/kg of pentobarbital sodium) due to the persistence of
95 severe aggressive behaviors,¹⁴ while 14 died for different organic causes. All the cats
96 were necropsied to exclude the presence of organic pathologies that could influence
97 their behavior. During the necropsy, the VNOs of all the cats were sampled, fixed in
98 10% buffered formalin (pH 7.4) for 48 hours and processed by routine methods for
99 histological analyses. VNOs were cut transversally respect their length in order to
100 observe both the NSE and the VNSE at the histological exam. Four- μ m-thick sections
101 were stained with haematoxylin and eosin for the histopathological analysis. Due to the
102 different roles played by the NSE and the VNSE side of the VNO,^{4,5} they were
103 considered as separated entities for the histopathological evaluation and the statistical
104 analysis. Inflammations were classified as acute or chronic, depending on the type of
105 cellular infiltrate and the duration of the process.^{15,16}

106 About all the subjects, data regarding the presence of aggressive
107 behaviors against humans or against the other cats were collected by means of a
108 questionnaire completed by the owners at the moment of the death.

109 Statistical analysis was performed using 9.4 SAS software (SAS Institute Inc.,
110 Cary, NC, USA). The Fisher exact test was used to investigate the significance of the
111 relationship between VNSE and NSE inflammation and the following parameters: sex,

112 age, presence of intraspecific aggression and presence of aggression against humans,
113 using the FREQ procedure. Statistical significance was based on a 5% (0.05)
114 significance level.

115

116 **Results**

117 *Histological analysis*

118 The VNSE was affected by inflammation in 11 of the 20 samples (55%) included in this
119 study, while 13/20 (65%) were affected by inflammation of the NSE. Overall, the VNO
120 was inflamed in 14/20 (70%) cats, and 10 of the 20 VNOs (50%) presented a concurrent
121 inflammation of the two epithelia. Of the 4 discordant cases, 3 were affected only in the
122 NSE and 1 only in the VNSE. Six of the 20 VNOs (30%) were not altered. Data
123 regarding VNO inflammation are illustrated in Table 1.

124 All the 14 vomeronasalitis were classified as chronic inflammations. Nine of
125 these lesions were also subclassified as follows: 6/20 (30%) were chronic-active
126 processes, 2/20 (10%) were pyogranulomatous inflammations, and 1/20 (5%) was a
127 VNO vasculitis. This latter affected the VNO of a cat dead for an effusive form of
128 Feline Infectious Peritonitis (FIP), and it was characterized by the infiltration of
129 inflammatory cells around blood vessels and vascular congestion. In the 5/20 (25%)
130 VNOs affected by a classic chronic process, the inflammatory infiltrate was mainly

131 composed by small lymphocytes, with a minor involvement of plasma cells and
132 macrophages (Figure 1A and 1B). The 6 chronic-active vomeronasalitis were
133 characterized by the same cellular infiltrate of the chronic ones, but with the concurrent
134 involvement of mature neutrophils, sign of the restarting of the inflammatory process.
135 In the two VNOs presenting pyogranulomatous inflammation, the cellular infiltrate was
136 composed by macrophages and neutrophils, often affected by degenerative processes
137 (Figure 1C). In chronic, chronic-active and pyogranulomatous lesions, inflammatory
138 cells were mainly infiltrated in the VNO soft tissue under the NSE and VNSE and
139 among the epithelial layers (Figure 1). In 4 VNOs, inflammatory cells arrived to mildly
140 infiltrate vomeronasal glands and nerves. The necrosis of the VNO epithelia was rarely
141 observed and commonly associated to the detachment of the necrotic cells into the
142 lumen (Figure 1B and 1D). Furthermore, the inflammation of VNSE and NSE was often
143 accompanied by the hydropic degeneration of the epithelial cells (Figure 1C).

144

145 *Behavioral data*

146 Of the 20 cats, 5 (25%) presented aggressive behaviors against the other cats and 8
147 (40%) against humans. Two of the 20 cats (10%) presented aggression both towards
148 cats and humans, while 9/20 subjects (45%) were not aggressive at all. Data regarding
149 aggressive behaviors are reported in Table 2.

150

151 *Statistical analysis*

152 The Fisher's exact test showed a statistically significant correlation between VNSE
153 inflammation and the presence of aggressive behaviors against cats ($P = 0.038$). No
154 correlation was observed between VNSE inflammation and sex, age and aggression
155 against humans ($P > 0.05$). However, the test showed a statistical trend between VNSE
156 inflammation and female cats ($P = 0.065$). No statistical significant correlation or trend
157 were instead observed between NSE inflammation and the other parameters included in
158 the statistical analysis.

159

160 **Discussion**

161 This study describes for the first time the feline vomeronasalitis and its possible
162 association to aggressiveness in cats. Our data showed that a large percentage of feline
163 VNOs can be affected by chronic inflammation, which can involve both the NSE and
164 VNSE. The statistical analysis showed that only the VNSE inflammation could be
165 associated to intercat aggression perceived by the owners.

166 The importance of the VNO in several aspects of animal behavior has been
167 widely demonstrated by the surgical removal of this structure.⁸ In fact, after VNO

168 ablation or making VNO inaccessible, animals showed marked deficits in social and
169 reproductive behavior.^{9,10,17,18,19} On the other hand, there are no studies in literature
170 focused on VNO **diseases** and VNO alterations have been rarely reported only as a
171 secondary finding in experimental condition.^{6,7} The presence of lymphatic nodules
172 under the vomeronasal epithelium has been described in tree shrews,⁶ while in rabbits it
173 has been observed the formation of VNO microabscesses as consequence of
174 experimental vehicle instillation in the nasal cavity.⁷

175 Due to the absence of bibliography about vomeronasalitis, we can draw a
176 parallel between our findings and feline chronic rhinitis, also considering the similar
177 position and histomorphology of the two structures.⁴ Chronic rhinitis is a common
178 finding in cats of any age, and it is mainly composed by a lymphoplasmacytic
179 infiltrate.^{15,16} A number of different causes can be responsible for the onset of this
180 condition, but the viral etiology is the more involved in this process.¹⁶ Bacterial
181 infections are also commonly identified during feline chronic rhinitis, but this finding is
182 normally considered a secondary consequence of the viral infection.¹⁶ The similar
183 anatomical position suggests that the feline chronic vomeronasalitis may be caused by
184 the same etiologies of the chronic rhinitis. A peculiar data of our study is that the feline
185 VNO was affected just by chronic processes, while neutrophils were involved only in
186 association to a pre-existing lymphocytic or macrophagic infiltrate. In feline rhinitis,
187 neutrophilic infiltrate is considered twice as common as the lymphocytic type, and it is

188 associated to acute and suppurative processes.¹⁶ In our study, we did not observe acute
189 suppurative vomeronasalitis, and this finding could maybe suggest some differences in
190 the immunity function between nasal and vomeronasal epithelium or in the entry
191 pathway of the etiological cause. In fact, the entry of the air in the VNO is regulated by
192 a pumping/suction mechanism that makes VNO inaccessible for the majority of the
193 time, differently from what happens in the nasal cavity.¹²

194 Even if a larger number of subjects should be investigated to verify the cause-
195 effect relationship between vomeronasalitis and intraspecific aggression, our data
196 showed that the inflammation of the VNSE was statistically correlated to intraspecific
197 aggression, while NSE inflammation was not correlated. As previously reported, VNSE
198 closely resembles the sensorial epithelium of the main olfactory system of the nasal
199 cavity.^{4,5} In humans, the chronic inflammation of the olfactory mucosa has been
200 associated to the reduction of odor perception, as a probable consequence of
201 inflammation mediators and cytokines effect on receptor neurons and nerves.²⁰ In fact,
202 the chronic exposure to tumor necrosis factor-alpha (TNF- α) leads to olfactory
203 dysfunction through reduction of neurons number, olfactory epithelium thickness and
204 nervous function in mice.^{21,22} TNF- α is a cytokine mainly produced by lymphocytes and
205 macrophages,²² cells that mainly composed the inflammatory infiltrate of the feline
206 VNOs analyzed in this study. Also another macrophagic and lymphocytic cytokine, the
207 interleukin 6 (IL-6), has been associated to human hyposmia.²³ Thus, it is authors'

208 opinion that the inflammatory microenvironment proposed as cause for humans and
209 mouse olfactory loss may also impair feline VNO during spontaneous vomeronasalitis.
210 On the contrary, NSE inflammation was not correlated with intraspecific aggression.
211 This data confirms that only the VNSE is responsible for pheromones detection, as
212 widely reported in literature.^{4,5,8} VNSE and NSE inflammations were not correlated to
213 aggression towards people, suggesting that probably in cat-human interactions other
214 factors (e.g. postural communication, vocalizations, owners' characteristics) play a
215 more crucial role.²⁴ On the contrary, our data suggest that chemical communication
216 plays a putative stronger role in intercat relationship than those factors involved in
217 human-cat interaction control.

218 Among feline behavioral alterations, intraspecific aggression is the most
219 common disorder.^{25,26} This condition can be fear-related, status- or conflict-related,
220 play-related, redirected or caused by the entry of a new subject into an pre-existing
221 group of cats.²⁶ However, until now, VNO diseases have been never proposed as
222 potential factor of intercat aggression. Our data seem to suggest that the inflammation
223 of this organ and its following dysfunction could lead to intraspecific communication
224 alterations causing intercat aggression. These data open a new way of investigation that
225 links pathology to behavioral medicine. Moreover, the following studies should
226 investigate the possible correlation between vomeronasalitis and the different kind of

227 aggressiveness, in order to evaluate possible dissimilarities between them or the
228 subjects involved in the social interactions (familiar or unfamiliar subjects).

229

230 **Conclusions**

231 To the best of our knowledge, this is the first study describing VNO diseases in cats or
232 in other species. Due to the pivotal role of this organ in animal behavior, this first
233 report reveals several clinical and behavioral implications of VNO pathological
234 changes. Even if a wider number of VNO samples are needed to draw firmer
235 conclusions, our data suggest that vomeronasalitis could be a cofactor in the onset of
236 intercat aggressive behavior.

237

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240 not-for-profit sectors.

241

242 **Conflict of interest statement**

243 The authors declare that there is no conflict of interest.

244

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246

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334 VNSE. Lymphocytes and plasma cells interspersed within nerves (arrowheads) and
335 vomeronasal glands (arrows). (haematoxylin and eosin stain, x 400).

336

337

Table 1 Histopathological findings in the vomeronasal organ of the 20 cats included in this study

Histopathological finding	VNO		VNSE		NSE	
	Number of cases	Percent	Number of cases	Percent	Number of cases	Percent
No alterations	6	30	9	45	7	35
Inflammation	14	70	11	55	13	65
- Chronic	5	25	3	15	5	25
- Chronic-active	6	30	5	25	5	25
- Pyogranulomatous	2	10	2	10	2	10
- Vasculitis	1	5	1	5	1	5

VNO = Vomeronasal organ; VNSE = Vomeronasal sensory epithelium; NSE = Non-sensory epithelium

Table 2 Presence of aggressive behaviors in the 20 cats included in this study

	Aggression towards cats		Aggression towards humans	
	Number of cases	Percent	Number of cases	Percent
Positive	5	25	8	40
Negative	15	75	12	60

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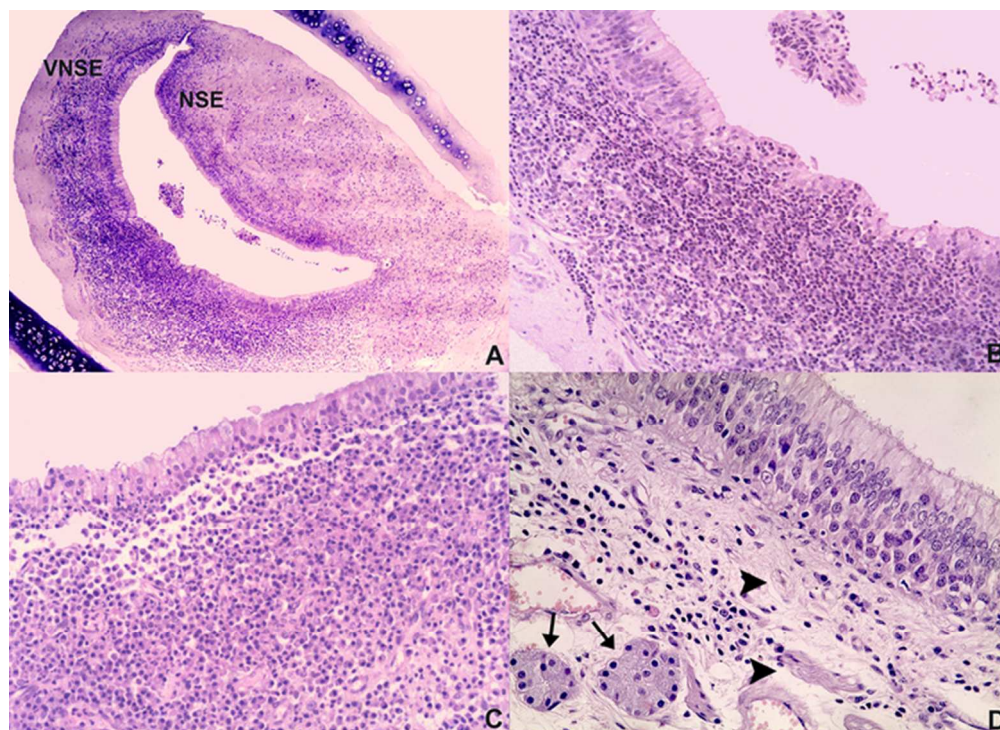


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