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

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.

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.

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
Cats or humans.

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.
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...
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 trully believe the topic is unique and raises important concerns regarding the link between deasease 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 dont have evidence to say vomeronasalitis is a fator 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 confidente 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 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 “diasease” 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
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 factor in the onset...”
  We followed reviewer’s suggestion.

- Line 165 “seems to be a factor 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 within behaviour therapy. Thus, when talking about aggression do prefer “behavior alteration”
  We rephrased in order to clarify this aspect.
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

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Abstract

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.

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.

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 cats or humans.

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

Up to now, evidences of VNO alteration have been rarely reported in literature. In 1974, Loo and Chin described the presence of lymphatic nodules in the VNO of tree shrews, but this finding was not considered a pathological change.\(^6\) More recently, microabscesses were observed in the VNO of rabbits after the instillation of experimental vehicles in the nasal cavity.\(^7\) No reports described instead spontaneous pathological changes of the VNO in other animals. In fact, only induced VNO
alterations have been reported, by several studies finding that the surgical removal of the VNO leads to the marked increase of deficits in social and reproductive behavior in various species\textsuperscript{8,9,10}

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

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

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

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

Statistical analysis was performed using 9.4 SAS software (SAS Institute Inc., Cary, NC, USA). The Fisher exact test was used to investigate the significance of the relationship between VNSE and NSE inflammation and the following parameters: sex,
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age, presence of intraspecific aggression and presence of aggression against humans, using the FREQ procedure. Statistical significance was based on a 5% (0.05) significance level.

Results

Histological analysis

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

All the 14 vomeronasalitis were classified as chronic inflammations. Nine of these lesions were also subclassified as follows: 6/20 (30%) were chronic-active processes, 2/20 (10%) were pyogranulomatous inflammations, and 1/20 (5%) was a VNO vasculitis. This latter affected the VNO of a cat dead for an effusive form of Feline Infectious Peritonitis (FIP), and it was characterized by the infiltration of inflammatory cells around blood vessels and vascular congestion. In the 5/20 (25%) VNOs affected by a classic chronic process, the inflammatory infiltrate was mainly
composed by small lymphocytes, with a minor involvement of plasma cells and
macrophages (Figure 1A and 1B). The 6 chronic-active vomeronasalitis were
characterized by the same cellular infiltrate of the chronic ones, but with the concurrent
involvement of mature neutrophils, sign of the restarting of the inflammatory process.
In the two VNOs presenting pyogranulomatous inflammation, the cellular infiltrate was
composed by macrophages and neutrophils, often affected by degenerative processes
(Figure 1C). In chronic, chronic-active and pyogranulomatous lesions, inflammatory
cells were mainly infiltrated in the VNO soft tissue under the NSE and VNSE and
among the epithelial layers (Figure 1). In 4 VNOs, inflammatory cells arrived to mildly
infiltrate vomeronal glands and nerves. The necrosis of the VNO epithelia was rarely
observed and commonly associated to the detachment of the necrotic cells into the
lumen (Figure 1B and 1D). Furthermore, the inflammation of VNSE and NSE was often
accompanied by the hydropic degeneration of the epithelial cells (Figure 1C).

Behavioral data

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

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

Discussion

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

The importance of the VNO in several aspects of animal behavior has been widely demonstrated by the surgical removal of this structure.\(^8\) In fact, after VNO
ablation or making VNO inaccessible, animals showed marked deficits in social and reproductive behavior. On the other hand, there are no studies in literature focused on VNO diseases and VNO alterations have been rarely reported only as a secondary finding in experimental condition. The presence of lymphatic nodules under the vomeronasal epithelium has been described in tree shrews, while in rabbits it has been observed the formation of VNO microabscesses as consequence of experimental vehicle instillation in the nasal cavity.

Due to the absence of bibliography about vomeronasalitis, we can draw a parallel between our findings and feline chronic rhinitis, also considering the similar position and histomorphology of the two structures. Chronic rhinitis is a common finding in cats of any age, and it is mainly composed by a lymphoplasmacytic infiltrate. A number of different causes can be responsible for the onset of this condition, but the viral etiology is the more involved in this process. Bacterial infections are also commonly identified during feline chronic rhinitis, but this finding is normally considered a secondary consequence of the viral infection. The similar anatomical position suggests that the feline chronic vomeronasalitis may be caused by the same etiologies of the chronic rhinitis. A peculiar data of our study is that the feline VNO was affected just by chronic processes, while neutrophils were involved only in association to a pre-existing lymphocytic or macrophagic infiltrate. In feline rhinitis, neutrophilic infiltrate is considered twice as common as the lymphocytic type, and it is
associated to acute and suppurative processes.\textsuperscript{16} In our study, we did not observe acute suppurative vomeronasalitis, and this finding could maybe suggest some differences in the immunity function between nasal and vomeronasal epithelium or in the entry pathway of the etiological cause. In fact, the entry of the air in the VNO is regulated by a pumping/suction mechanism that makes VNO inaccessible for the majority of the time, differently from what happens in the nasal cavity.\textsuperscript{12}

Even if a larger number of subjects should be investigated to verify the cause-effect relationship between vomeronasalitis and intraspecific aggression, our data showed that the inflammation of the VNSE was statistically correlated to intraspecific aggression, while NSE inflammation was not correlated. As previously reported, VNSE closely resembles the sensorial epithelium of the main olfactory system of the nasal cavity.\textsuperscript{4,5} In humans, the chronic inflammation of the olfactory mucosa has been associated to the reduction of odor perception, as a probable consequence of inflammation mediators and cytokines effect on receptor neurons and nerves.\textsuperscript{20} In fact, the chronic exposure to tumor necrosis factor-alpha (TNF-\(\alpha\)) leads to olfactory dysfunction through reduction of neurons number, olfactory epithelium thickness and nervous function in mice.\textsuperscript{21,22} TNF-\(\alpha\) is a cytokine mainly produced by lymphocytes and macrophages,\textsuperscript{22} cells that mainly composed the inflammatory infiltrate of the feline VNOs analyzed in this study. Also another macrophagic and lymphocytic cytokine, the interleukin 6 (IL-6), has been associated to human hyposmia.\textsuperscript{23} Thus, it is authors’
opinion that the inflammatory microenvironment proposed as cause for humans and mouse olfactory loss may also impair feline VNO during spontaneous vomeronasalitis. On the contrary, NSE inflammation was not correlated with intraspecific aggression. This data confirms that only the VNSE is responsible for pheromones detection, as widely reported in literature.\textsuperscript{4,5,8} VNSE and NSE inflammations were not correlated to aggression towards people, suggesting that probably in cat-human interactions other factors (e.g. postural communication, vocalizations, owners’ characteristics) play a more crucial role.\textsuperscript{24} On the contrary, our data suggest that chemical communication plays a putative stronger role in intercat relationship than those factors involved in human-cat interaction control.

Among feline behavioral alterations, intraspecific aggression is the most common disorder.\textsuperscript{25,26} This condition can be fear-related, status- or conflict-related, play-related, redirected or caused by the entry of a new subject into an pre-existing group of cats.\textsuperscript{26} However, until now, VNO diseases have been never proposed as potential factor of intercat aggression. Our data seem to suggest that the inflammation of this organ and its following dysfunction could lead to intraspecific communication alterations causing intercat aggression. These data open a new way of investigation that links pathology to behavioral medicine. Moreover, the following studies should investigate the possible correlation between vomeronasalitis and the different kind of
aggressiveness, in order to evaluate possible dissimilarities between them or the 
subjects involved in the social interactions (familiar or unfamiliar subjects).

Conclusions

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

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not-for-profit sectors.

Conflict of interest statement

The authors declare that there is no conflict of interest.
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Figure 1 Cat, vomeronasal organ. (A) Feline chronic vomeronasalitis affecting the vomeronasal sensory epithelium (VNSE) and the non-sensory epithelium (NSE). (haematoxylin and eosin stain, x 40). (B) Feline chronic vomeronasalitis affecting VNSE. Inflammatory infiltrate is mainly composed by lymphocytes; macrophage and plasma cells are also present. VNSE is totally disrupted by the presence of inflammatory cells and clusters of necrotic epithelial cells are observable into the VNO lumen. (haematoxylin and eosin stain, x 200). (C) Feline pyogranulomatous vomeronasalitis affecting VNSE. Macrophages and neutrophils massively infiltrate the soft tissue under VNSE, which is reduced in thickness and partially necrotic. (haematoxylin and eosin stain, x 200). (D) Feline chronic vomeronasalitis affecting VNSE. Lymphocytes and plasma cells interspersed within nerves (arrowheads) and vomeronasal glands (arrows). (haematoxylin and eosin stain, x 400).
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Keywords: aggression, behavior, cat, inflammation, pathology, pheromones, vomeronasal organ

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Introduction

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

Up to now, evidences of VNO alteration have been rarely reported in literature. In 1974, Loo and Chin described the presence of lymphatic nodules in the VNO of tree shrews, but this finding was not considered a pathological change.\textsuperscript{6} More recently, microabscesses were observed in the VNO of rabbits after the instillation of experimental vehicles in the nasal cavity.\textsuperscript{7} No reports described instead spontaneous pathological changes of the VNO in other animals. In fact, only induced VNO
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Considering the absence of substantial literature about VNO diseases and the pivotal role of the VNO in the social life and communication of the cat, the aim of this study was to evaluate if this organ can be affected by inflammatory alterations, and if these conditions can be associated with feline aggressive behaviors.

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

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

Statistical analysis was performed using 9.4 SAS software (SAS Institute Inc., Cary, NC, USA). The Fisher exact test was used to investigate the significance of the relationship between VNSE and NSE inflammation and the following parameters: sex,
age, presence of intraspecific aggression and presence of aggression against humans, using the FREQ procedure. Statistical significance was based on a 5% (0.05) significance level.

Results

Histological analysis

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

All the 14 vomeronasalitis were classified as chronic inflammations. Nine of these lesions were also subclassified as follows: 6/20 (30%) were chronic-active processes, 2/20 (10%) were pyogranulomatous inflammations, and 1/20 (5%) was a VNO vasculitis. This latter affected the VNO of a cat dead for an effusive form of Feline Infectious Peritonitis (FIP), and it was characterized by the infiltration of inflammatory cells around blood vessels and vascular congestion. In the 5/20 (25%) VNOs affected by a classic chronic process, the inflammatory infiltrate was mainly
composed by small lymphocytes, with a minor involvement of plasma cells and macrophages (Figure 1A and 1B). The 6 chronic-active vomeronasalitis were characterized by the same cellular infiltrate of the chronic ones, but with the concurrent involvement of mature neutrophils, sign of the restarting of the inflammatory process. In the two VNOs presenting pyogranulomatous inflammation, the cellular infiltrate was composed by macrophages and neutrophils, often affected by degenerative processes (Figure 1C). In chronic, chronic-active and pyogranulomatous lesions, inflammatory cells were mainly infiltrated in the VNO soft tissue under the NSE and VNSE and among the epithelial layers (Figure 1). In 4 VNOs, inflammatory cells arrived to mildly infiltrate vomeronasal glands and nerves. The necrosis of the VNO epithelia was rarely observed and commonly associated to the detachment of the necrotic cells into the lumen (Figure 1B and 1D). Furthermore, the inflammation of VNSE and NSE was often accompanied by the hydropic degeneration of the epithelial cells (Figure 1C).

Behavioral data

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

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

Discussion

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

The importance of the VNO in several aspects of animal behavior has been widely demonstrated by the surgical removal of this structure.\textsuperscript{8} In fact, after VNO
ablation or making VNO inaccessible, animals showed marked deficits in social and reproductive behavior.\textsuperscript{9,10,17,18,19} On the other hand, there are no studies in literature focused on VNO \textit{diseases} and VNO alterations have been rarely reported only as a secondary finding in experimental condition.\textsuperscript{6,7} The presence of lymphatic nodules under the vomeronasal epithelium has been described in tree shrews,\textsuperscript{6} while in rabbits it has been observed the formation of VNO microabscesses as consequence of experimental vehicle instillation in the nasal cavity.\textsuperscript{7}

Due to the absence of bibliography about vomeronasalitis, we can draw a parallel between our findings and feline chronic rhinitis, also considering the similar position and histomorphology of the two structures.\textsuperscript{4} Chronic rhinitis is a common finding in cats of any age, and it is mainly composed by a lymphoplasmacytic infiltrate.\textsuperscript{15,16} A number of different causes can be responsible for the onset of this condition, but the viral etiology is the more involved in this process.\textsuperscript{16} Bacterial infections are also commonly identified during feline chronic rhinitis, but this finding is normally considered a secondary consequence of the viral infection.\textsuperscript{16} The similar anatomical position suggests that the feline chronic vomeronasalitis may be caused by the same etiologies of the chronic rhinitis. A peculiar data of our study is that the feline VNO was affected just by chronic processes, while neutrophils were involved only in association to a pre-existing lymphocytic or macrophagic infiltrate. In feline rhinitis, neutrophilic infiltrate is considered twice as common as the lymphocytic type, and it is
associated to acute and suppurative processes.\textsuperscript{16} In our study, we did not observe acute suppurative vomeronasalitis, and this finding could maybe suggest some differences in the immunity function between nasal and vomeronasal epithelium or in the entry pathway of the etiological cause. In fact, the entry of the air in the VNO is regulated by a pumping/suction mechanism that makes VNO inaccessible for the majority of the time, differently from what happens in the nasal cavity.\textsuperscript{12}

Even if a larger number of subjects should be investigated to verify the cause-effect relationship between vomeronasalitis and intraspecific aggression, our data showed that the inflammation of the VNSE was statistically correlated to intraspecific aggression, while NSE inflammation was not correlated. As previously reported, VNSE closely resembles the sensorial epithelium of the main olfactory system of the nasal cavity.\textsuperscript{4,5} In humans, the chronic inflammation of the olfactory mucosa has been associated to the reduction of odor perception, as a probable consequence of inflammation mediators and cytokines effect on receptor neurons and nerves.\textsuperscript{20} In fact, the chronic exposure to tumor necrosis factor-alpha (TNF-\(\alpha\)) leads to olfactory dysfunction through reduction of neurons number, olfactory epithelium thickness and nervous function in mice.\textsuperscript{21,22} TNF-\(\alpha\) is a cytokine mainly produced by lymphocytes and macrophages,\textsuperscript{22} cells that mainly composed the inflammatory infiltrate of the feline VNOs analyzed in this study. Also another macrophagie and lymphocytic cytokine, the interleukin 6 (IL-6), has been associated to human hyposmia.\textsuperscript{23} Thus, it is authors’
opinion that the inflammatory microenvironment proposed as cause for humans and mouse olfactory loss may also impair feline VNO during spontaneous vomeronasalitis. On the contrary, NSE inflammation was not correlated with intraspecific aggression. This data confirms that only the VNSE is responsible for pheromones detection, as widely reported in literature. VNSE and NSE inflammations were not correlated to aggression towards people, suggesting that probably in cat-human interactions other factors (e.g. postural communication, vocalizations, owners’ characteristics) play a more crucial role. On the contrary, our data suggest that chemical communication plays a putative stronger role in intercat relationship than those factors involved in human-cat interaction control.

Among feline behavioral alterations, intraspecific aggression is the most common disorder. This condition can be fear-related, status- or conflict-related, play-related, redirected or caused by the entry of a new subject into an pre-existing group of cats. However, until now, VNO diseases have been never proposed as potential factor of intercat aggression. Our data seem to suggest that the inflammation of this organ and its following dysfunction could lead to intraspecific communication alterations causing intercat aggression. These data open a new way of investigation that links pathology to behavioral medicine. Moreover, the following studies should investigate the possible correlation between vomeronasalitis and the different kind of
aggressiveness, in order to evaluate possible dissimilarities between them or the subjects involved in the social interactions (familiar or unfamiliar subjects).

Conclusions

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

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Conflict of interest statement

The authors declare that there is no conflict of interest.
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Figure legend

**Figure 1** Cat, vomeronasal organ. (A) Feline chronic vomeronasalitis affecting the vomeronasal sensory epithelium (VNSE) and the non-sensory epithelium (NSE). (haematoxylin and eosin stain, x 40). (B) Feline chronic vomeronasalitis affecting VNSE. Inflammatory infiltrate is mainly composed by lymphocytes; macrophage and plasma cells are also present. VNSE is totally disrupted by the presence of inflammatory cells and clusters of necrotic epithelial cells are observable into the VNO lumen. (haematoxylin and eosin stain, x 200). (C) Feline pyogranulomatous vomeronasalitis affecting VNSE. Macrophages and neutrophils massively infiltrate the soft tissue under VNSE, which is reduced in thickness and partially necrotic. (haematoxylin and eosin stain, x 200). (D) Feline chronic vomeronasalitis affecting VNSE. Lymphocytes and plasma cells interspersed within nerves (arrowheads) and vomeronasal glands (arrows). (haematoxylin and eosin stain, x 400).
Table 1 Histopathological findings in the vomeronasal organ of the 20 cats included in this study

<table>
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<th>Histopathological finding</th>
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<th>VNSE</th>
<th>NSE</th>
</tr>
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<tbody>
<tr>
<td>No alterations</td>
<td>6</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>- Chronic</td>
<td>5</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>- Chronic-active</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>- Pyogranulomatous</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>- Vasculitis</td>
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<td>1</td>
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</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Aggression towards cats</th>
<th></th>
<th>Aggression towards humans</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of cases</td>
<td>Percent</td>
<td>Number of cases</td>
<td>Percent</td>
</tr>
<tr>
<td>Positive</td>
<td>5</td>
<td>25</td>
<td>8</td>
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</tr>
<tr>
<td>Negative</td>
<td>15</td>
<td>75</td>
<td>12</td>
<td>60</td>
</tr>
</tbody>
</table>
Figure 1 Cat, vomeronasal organ. (A) Feline chronic vomeronasalitis affecting the vomeronasal sensory epithelium (VNSE) and the non-sensory epithelium (NSE). (haematoxylin and eosin stain, x 40). (B) Feline chronic vomeronasalitis affecting VNSE. Inflammatory infiltrate is mainly composed by lymphocytes; macrophage and plasma cells are also present. VNSE is totally disrupted by the presence of inflammatory cells and clusters of necrotic epithelial cells are observable into the VNO lumen. (haematoxylin and eosin stain, x 200). (C) Feline pyogranulomatous vomeronasalitis affecting VNSE. Macrophages and neutrophils massively infiltrate the soft tissue under VNSE, which is reduced in thickness and partially necrotic. (haematoxylin and eosin stain, x 200). (D) Feline chronic vomeronasalitis affecting VNSE. Lymphocytes and plasma cells interspersed within nerves (arrowheads) and vomeronasal glands (arrows). (haematoxylin and eosin stain, x 400).

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