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Characterization of oral pain in cats after dental extractions in a multidisciplinary approach

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Résumé

Les maladies bucco-dentaires sont fréquemment rapportées en médecine vétérinaire et le traitement généralement nécessite l'extraction des dents. Cependant, la procédure est invasive et une évaluation à long terme ainsi qu'une gestion de la douleur sont nécessaires. En médecine vétérinaire, les opioïdes, les blocs anesthésiques locaux et les anti-inflammatoires non stéroïdiens sont administrés en intervention analgésique péri-opératoire. Par exemple, la buprénorphine est un opioïde analgésique puissant, hautement lipophile, et est principalement utilisé pour traiter la douleur aiguë. La buprénorphine est souvent administrée dans le cadre d'une analgésie multimodale.

Les signes comportementaux de la douleur induite par les maladies bucco-dentaires n'ont pas été systématiquement étudiés chez les chats, et les connaissances actuelles sont principalement basées sur des preuves anecdotiques ou des études réalisées chez d'autres espèces. On ignore comment les maladies bucco-dentaires et le traitement (c'est-à-dire l'extraction dentaire) peuvent affecter la prise alimentaire péri-opératoire, les scores de douleur, les besoins analgésiques supplémentaires et les comportements chez les chats. En outre, il serait important de savoir si l'échelle de douleur basée sur l'expression faciale (Feline Grimace Scale: FGS) pourrait également être utilisée pour l'évaluation de la douleur buccale.

Les objectifs du projet étaient 1) d'identifier les comportements spécifiques associés aux maladies bucco-dentaires en utilisant une évaluation par vidéo, et de les corréler aux scores de la douleur en temps réel, 2) d'évaluer l'impact des maladies bucco-dentaires et de la douleur sur la prise alimentaire et les comportements liés à l'alimentation, 3) de déterminer les effets du traitement des maladies bucco-dentaires sur le comportement, les scores de la douleur et la prise alimentaire, 4) d'évaluer la fiabilité inter-évaluateurs du FGS et 5) pour évaluer l'efficacité analgésique et les événements indésirables d'une formulation à haute concentration de formulation de chlorhydrate de buprénorphine (Simbadol, 1,8 mg / mL) en comparaison avec une formulation standard de chlorhydrate

de buprénorphine (Vetergesic, 0,3 mg / mL) dans le cadre d'un schéma multimodal chez les chats subissant des extractions dentaires. Les hypothèses étaient que 1) des comportements spécifiques pourraient être identifiés et corrélés aux scores de la douleur en temps réel, 2) les chats atteints d'une maladie bucco-dentaire sévère auraient une consommation alimentaire plus faible et des scores de douleur plus élevés et nécessiteraient une analgésie de secours comparativement aux chats qui ne sont pas / minimalement atteints par une maladie bucco-dentaire, 3) le traitement des maladies bucco-dentaires réduirait la prévalence des comportements spécifiques ainsi que les scores de douleur et améliorerait la consommation alimentaire de ces animaux, 4) les scores FGS notés par différents évaluateurs seraient fiables et 5) Simbadol et Vetergesic produiraient tous deux des scores de douleur postopératoire, des événements indésirables, ainsi que le moment et la prévalence de l'analgésie de secours similaires lors de l'utilisation du Glasgow Composite Measure Pain Scale-Feline (CMPS-F).

Le projet a été divisé en deux études et quatre articles (étude 1: articles 1 à 3, étude 2: article 4): 1) article sur le score de la douleur, les besoins en analgésie de secours et la quantité de nourriture ingérée chez les chats subissant un traitement oral, 2) article sur les comportements spécifiques induits par la douleur liés à la douleur buccale chez les chats sous traitement oral, 3) article sur la fiabilité inter-évaluateurs de la FGS chez les chats sous traitement oral, et 4) comparaison détaillée de l'efficacité analgésique de deux schémas posologiques en utilisant deux concentrations différentes de buprénorphine chez les chats subissant des extractions dentaires.

Dans le premier article, vingt-quatre chats ont été répartis également en deux groupes: un groupe qui représente des maladies bucco-dentaires légères (traitement dentaire minimal) et un autre sévères (extractions dentaires multiples) sur la base d'un système de notation dentaire qui impliquait le nombre et l'emplacement de l'extraction des dents et hospitalisés pendant 7 jours (admission au jour 0, examen bucco-dentaire, radiographies et traitement sous anesthésie générale le jour 1 et sortie le jour 6). Pendant l'hospitalisation, les scores de douleur basés sur l'échelle composite de Glasgow (CMPS-F), la prévalence de l'analgésie de secours (CMPS-F ≥ 5/20), la prise d'aliments secs et mous (%) pendant 3 minutes et 2 heures, l'apport quotidien d'aliments mous et les

cytokines inflammatoires sériques ont été analysés. Dans le deuxième article, les chats ont été filmés à distance pendant 10 min tout au long de l'étude à différents moments (au total 36h d'enregistrement vidéo). Les vidéos se composaient de quatre parties soit les comportements généraux, de jeu, d'alimentation et post-alimentation. La durée et la fréquence des différents comportements basés sur un éthogramme ont été analysées. Dans le troixième article, quatre-vingt-onze captures d'images (c'est-à-dire des captures d'écran) à partir de vidéos filmées aux jours 1 (postopératoire 6 heures) et 6 pour l'article 2 et des vidéos filmées avant / après l'analgésie de sauvetage ont été incluses. Le FGS comprend cinq unités d'action (AU): les yeux, les oreilles, le museau, les moustaches et la position de la tête. Les scores FGS des images ont été évalués indépendamment par quatre évaluateurs en aveugle. La fiabilité inter-évaluateurs de chaque score AU et FGS total et l'effet de la présence du soignant ont été évalués.

Dans l'étude 2 (article 4), vingt-trois chats subissant des extractions dentaires ont été inclus. Les chats ont reçu aléatoirement soit Simbadol (1.8 mg/mL; 0.24 mg/kg SC, toutes les 24 heures, n = 11) ou Vetergesic (0.3 mg/mL; 0.02 mg/kg IM, toutes les 8 h, n = 12) tout au long de l'étude. Ils ont été admis au jour 0, ont subi un examen oral, des radiographies et un traitement sous anesthésie générale le jour 1 et ont été libérés le jour 4. La sédation et la douleur ont été évaluées à l'aide de l'échelle visuelle analogique interactive dynamique (jour 1) et CMPS-F, respectivement. Les scores de sédation, de douleur et la prévalence de l'analgésie de secours (CMPS-F ≥ 5/20) et du ressentiment (défini comme tout type de comportement d'évitement associé à l'aversion pour l'administration de médicaments) ont été analysés statistiquement.

Les études ont montré que les scores de la douleur et la prévalence de l'analgésie de secours étaient significativement élevés, ainsi que les apports d'aliments secs et mous étaient significativement diminués chez les chats atteints d'une maladie grave par rapport à ceux présentant une maladie légère. De surcroit, la maladie buccale influence les cytokines inflammatoires et induit des comportements. Par ailleurs, Le FGS est un outil fiable pour l’évaluation de la douleur buccale et n’est pas affecté par la présence du soignant. En outre, les scores de la douleur et la prévalence de l'analgésie de secours chez les chats auxquels Simbadol a été administré n'étaient pas significativement

différents de ceux administrés par Vetergesic. De plus, certains chats administrés par Vetergesic ont développé un ressentiment à l'égard de l'administration du médicament, qui n'était pas significativement différent de ceux administrés par Simbadol.

Une analgésie à long terme est nécessaire après des extractions dentaires chez les chats atteints d'une maladie bucco-dentaire sévère. La diminution de l'apport alimentaire et les comportements spécifiques identifiés dans les études pourraient être utilisés pour différencier entre les chats douloureux des chats indolores dans la pratique clinique. Le FGS est un outil fiable pour l'évaluation de la douleur chez les chats subissant des extractions dentaires. Simbadol a produit des effets analgésiques similaires à Vetergesic sans induire un ressentiment pendant l'administration du médicament.

Mots-clés : Analgésie, Analyse vidéo, Buprénorphine, Comportement, Dentisterie, Douleur, Évaluation de la douleur, Expression faciale, Félin, Nutrition

Abstract

Oral disease is one of the most commonly reported diseases in veterinary medicine, and tooth extractions are commonly required as the treatment. The procedure, however, is invasive, and long-term pain management is necessary. In veterinary medicine, opioids, local anesthetic blocks and nonsteroidal anti-inflammatory drugs are administered as perioperative analgesic intervention.

Behavioral signs of oral disease-induced pain have not been systematically investigated in cats, and the current knowledge is mostly based on anecdotal evidence or studies performed in other species. It is not known how oral disease and the treatment (i.e. tooth extractions) can affect perioperative food intake, pain scores, additional analgesic requirements and behaviors in cats. Also, it is not known if a facial expression-based pain scale (Feline Grimace Scale: FGS) could be used for oral pain assessment as well.

The objectives of this PhD program were: 1) to identify the specific behaviors associated with oral disease by using video assessment, and to verify their correlation with the real-time pain scores, 2) to assess the impact of oral disease and pain on food intake and feeding-related behaviors, 3) to determine the effects of oral disease treatment on behavior, pain scores and food intake, 4) to assess the inter-rater reliability of the FGS in cats undergoing dental extractions and 5) to evaluate the analgesic efficacy and adverse events of a high-concentration formulation of buprenorphine hydrochloride formulation (Simbadol, 1.8 mg/mL) in comparison with a standard buprenorphine hydrochloride formulation (Vetergesic, 0.3 mg/mL) as part of a multimodal regimen in cats undergoing dental extractions. The hypotheses were: 1) specific behaviors associated with oral disease would be identified and correlated with real-time pain scores, 2) cats with severe oral disease would have lower food intake and higher pain scores, and require rescue analgesia when compared with cats with no/minimal oral disease, 3) treatment of oral disease would reduce the prevalence of specific behaviors and pain scores and improve food consumption of these animals, 4) the FGS scores scored by different raters would be reliable in cats undergoing dental extractions and 5) both Simbadol and Vetergesic would produce similar postoperative pain scores, adverse events and timing and

prevalence of rescue analgesia when using the Glasgow Composite Measure Pain Scale-Feline (CMPS-F).

The project was divided into two studies and four articles (study 1: articles 1-3, study 2: article 4): 1) investigation of pain scores, rescue analgesia requirements and the amount of food intake in cats undergoing oral treatment, 2) investigation of the pain-induced specific behaviors related to oral pain in cats undergoing oral treatment, 3) investigation of inter-rater reliability of FGS in cats undergoing oral treatment, and 4) comparison of the analgesic efficacy of two dosage regimens using two different concentrations of buprenorphine in cats undergoing dental extractions.

In article 1, twenty-four cats were equally divided into minimal (minimal dental treatment) or severe (multiple dental extractions) oral disease groups based on a dental scoring system which involved the number and location of teeth extraction and hospitalized for 7 days (admission on day 0, oral examination, radiographs and treatment under general anesthesia on day 1 and discharge on day 6). During hospitalization, pain scores based on CMPS-F, the prevalence of rescue analgesia (CMPS-F $\geq$ 5/20), dry and soft food intake (%) during periods of 3 minutes and 2 hours, daily soft food intake and serum inflammatory cytokines were analyzed and compared. In article 2, cats were filmed remotely for 10 min throughout the study at different time points (total of 36h of video recording). The videos consisted of four parts namely general, playing, feeding and post-feeding behaviors. The duration and frequency of different behaviors based on an ethogram were analyzed. In article 3, ninety-one image captures (i.e. screenshots) from videos filmed at days 1 (postoperative 6 hours) and 6 for article 2 and videos filmed before/after rescue analgesia were included. The FGS comprises five action units (AU): eyes, ears, muzzle, whiskers and head position. The FGS scores of the images were independently scored by four blinded raters. Inter-rater reliability of each AU and total FGS scores and the effect of the caregiver's presence were evaluated.

In study 2 (article 4), twenty-three cats undergoing tooth extractions were included. Cats randomly received either Simbadol (1.8 mg/mL; 0.24 mg/kg SC, every 24h, n = 11) or Vetergesic (0.3 mg/mL; 0.02 mg/kg IM, every 8h, n = 12) throughout the study. They were admitted on day 0, underwent oral examination, radiographs and treatment under general

anesthesia on day 1 and discharged on day 4. Sedation and pain were scored using the dynamic interactive visual analog scale (day 1) and CMPS-F, respectively. Sedation and pain scores and the prevalence of rescue analgesia (CMPS-F $\geq$ 5/20) and resentment (defined as any type of escape behavior associated with aversion to drug administration) were analyzed.

The studies found that the pain scores and the prevalence of rescue analgesia were significantly increased, and dry and soft food intakes were significantly decreased in cats with severe disease when compared with those with minimal disease, and the oral disease influences inflammatory cytokines and induces the specific behaviors. FGS is a reliable tool for the assessment of oral pain and is not affected by the caregiver's presence. Pain scores and the prevalence of rescue analgesia in cats administered Simbadol were not significantly different from those administered Vetergesic, and some cats administered Vetergesic developed resentment to the administration of the drug, which was not significantly different from those administered Simbadol.

Long-term analgesia is required after dental extractions in cats with severe oral disease. A decrease in food intake and specific behaviors identified in the studies could be used to differentiate painful versus pain-free cats in clinical practice. The FGS is a reliable tool for pain assessment in cats undergoing dental extractions. Simbadol produced similar analgesic effects to Vetergesic without resentment during drug administration.

Keywords:

Analgesia, Behavior, Buprenorphine, Dentistry, Facial expression, Feline, Nutrition, Pain, Pain assessment, Video analysis

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List of Abbreviations

AMPA: Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

AU: Action unit

AVDC: American Veterinary Dental College

CGRP: Calcitonin gene-related peptide

CHUV: Centre hospitalier universitaire vétérinaire

CI: Confidence interval

CMPS-F: Glasgow Composite Measure Pain Scale-Feline

COPS-C/F: Composite Oral and Maxillofacial Pain Scale-Canine/Feline

COX: Cyclooxygenase

CRI: Constant rate infusion

C1, C2: Cervical 1, 2

DIVAS: Dynamic and interactive visual analog scale

DJD: Degenerative joint disease

FCGS: Feline chronic gingivostomatitis

FDA: Food and Drug Administration

FGS: Feline Grimace Scale

FOPS: Feline orofacial pain syndrome

GABA: γ-aminobutyric acid

IASP: International Association for the Study of Pain

ICC: Intraclass correlation coefficients

IFN: Interferon

IL: Interleukin

IM: Intramuscularly

IV: Intravenously

KC: Keratinocyte chemoattractant

LPS: Lipopolysaccharides

MCP: Monocyte chemoattractant protein

MCPS: UNESP-Botucatu Multidimensional Composite Pain Scale

MSCs: Mesenchymal stem cells

NK: Neurokinin

NMDA: N-methyl-D-aspartate

NSAIDs: Non-steroidal anti-inflammatory drugs

OHRQoL: Oral health-related quality of life

OTM: Oral transmucosal

PAG: Periaqueductal gray

PG: Prostaglandin

PO: Per os

PrV: Principal sensory nucleus

QoL: Quality of life

RANTES: Regulated on activation-normal T cell expressed and secreted

RVM: Ventromedial medulla

SC: Subcutaneously

SCF: Stem cell factor

SD: Standard deviation

SEM: Standard error of the mean

SDF: Stromal cell-derived factor

sFAS: Soluble free acid synthesis

SpV: Spinal nucleus

SP: Substance P

TG: Trigeminal ganglion

TNF: Tumor necrosis factor

TRP: Transient receptor potential

TRPA1: Transient receptor potential ankyrin 1

TRPM8: Transient receptor potential M member 8

TRPV1: Transient receptor potential vanilloid 1

TRPV2: Transient receptor potential vanilloid 2

TSNC: Trigeminal sensory nuclear complex

Vc: Spinal nucleus caudalis

Vi: Spinal nucleus interpolaris

Vo: Spinal nucleus oralis

VPM: Posterior ventromedial thalamus

5-HT: Serotonin

Dedication

To my parents,

I could not have achieved all that I have personally, academically, and professionally without your love and support.

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Introduction

According to the International Association for the Study of Pain (IASP), pain is defined as “an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage”, which requires comprehensive and ongoing assessment and effective management (1). Also, IASP recently suggested the new definition of pain as “a distressing experience associated with actual or potential tissue damage with sensory, emotional, cognitive, and social components” (2). The modification of some parts of the previous definition emphasizes that pain is an experience that is severer than “unpleasant” and significantly impacts social relationships.

Oral disease including periodontal disease is one of the most common diseases in both humans and small animals (3-7). However, studies on this subject report the use of local anesthetic techniques to reduce anesthetic requirements and to provide intraoperative analgesia in dogs and cats (8,9). Most of the clinical signs associated with the oral disease have been described only by review articles, textbooks and expert opinion. The dental pain-related specific behaviors and the amount of food intake has never been thoroughly investigated.

This literature review focuses on reporting the perspective of the current knowledge regarding oral pain in cats. The review is consisted of five parts. The first and second parts are intended to give an overview of the etiology of oral pain in humans and cats. The third part is about the dental pain pathway. The fourth part describes the pain assessment, and the fifth part will explore the strategy of pain management. Although the Ph.D. program was performed in cats, this literature review will also show the results of the studies in other species including dogs.

1. Dental disease and its impact on humans

Dental disease is a common cause of human medical visits (3). The disorder involves dental caries and periodontal disease. Previous data indicate that there were 2 million

visits to the emergency department associated with dental problems, and the personal health care expenditures for dental care was approximately $130 billion per year in the United States (4).

1.1 Dental caries

Dental caries is one of the most common preventable and chronic diseases. In children aged 5 to 17, dental caries is 5 times and 7 times more common than asthma and hay fever, respectively (5). The prevalence of dental caries is reported as more than 50% (5), and the prevalence increases with age; 51.6%, 77.9% and 84.7% for children aged 5 to 9, 17 and 18, respectively had at least one carious lesion or treated primary or permanent teeth in the United States (5).

Dental caries results from an ecological imbalance in the physiological equilibrium between tooth minerals and oral microbial biofilms. Endogenous bacteria in the biofilm produce weak acids as an acidic by-product from the bacterial fermentation of dietary carbohydrates including soda, sweets and salty snacks. The acid decreases the local pH, which results in the demineralization of tooth tissues and creates cavitation in the tooth. The demineralization can be reversed by calcium phosphate and fluoride intake. Whether dental caries progresses or reverses depends on the relation between demineralization and remineralization. Remineralization frequently occurs when the pH of biofilm is restored by saliva, which works as a buffer. The remineralized areas received calcium and phosphates from saliva, and the areas have a higher fluoride concentration and less microporous enamel structure than the demineralized structures. In adults, in addition to the dietary carbohydrate intake, illicit drugs and some prescribed medicines would increase the risk of enamel erosion and caries formation. For example, some drugs including opioids, antihistamines and proton pump inhibitors cause dry mouth by the decrease of saliva and some medications including antacids and cough syrups/drops that contain sugar (3).

The recession of the gingiva resulting from poor oral hygiene leads to exposure of the juncture of the crown with the root surface, and this area retains dental plaque and

develops dental caries. Inadequate salivary flow, the presence of cariogenic bacteria including *Streptococci* or *Lactobacilli mutans* and insufficient fluoride exposure, gingival recession, immunological components, need for special health care, socioeconomic status and genetic factors are considered as the risk factors of dental caries.

1.2 Periodontal disease

Periodontal disease is also a common disease, and approximately 50% of adults in the United States have periodontal disease (6). Similar to dental caries, periodontal diseases are caused by infections from bacteria in the biofilm (i.e. dental plaque) formed on oral surfaces. Periodontal disease is divided into two stages, gingivitis and periodontitis. Gingivitis is an inflammation of the gum characterized by a change from normal pink to red, swelling and bleeding, and the tissue is often sensitive and fragile. These changes are occurred by an accumulation of biofilm along the gingival margins and the inflammatory response from the immunity system to the release of destructive bacterial products. If tooth brushing and flossing are performed appropriately to remove the plaque, the early stage of gingivitis is reversible. Periodontitis is caused by an inflammation of soft tissues and the destruction of supporting structures including periodontal ligaments and bones, and the prevalence increases with age. Although the presence of gingivitis is not necessary, the gingivitis-related biofilm often seeds the subgingival plaque. The destruction of the periodontal ligament and bone creates a pocket between the tooth and adjacent tissues, and the pockets become the space for the accumulation of subgingival plaque. Previous studies revealed that more than 60% of adults aged greater than 25 years have at least 2 mm or more loss of attachment (5,6), and the prevalence of severe loss of attachment increases by age. At all ages, males are more likely than females to have severe loss of attachment (> 6 mm) in at least one tooth, and the prevalence is also higher in people with low socioeconomic when compared with those with high socioeconomic (5,6). Other modifiable risk factors are reported as smoking, poorly controlled diabetes, obesity, osteoporosis, low dietary calcium and vitamin D and stress (10).

1.3 Impact of oral disease

1.3.1. Oral health and systemic diseases

Oral disease is associated with not only local but also systemic problems including cardiovascular and respiratory diseases, oral and colorectal cancer, diabetes mellitus, Alzheimer's disease and adverse pregnancy outcomes (11-38). Oral bacteria can cause local inflammation, and it can also contribute to systemic inflammation through the release of toxins and/or leakage of microbial products to the bloodstream.

A meta-analysis that involved five prospective cohort studies revealed that people with periodontal disease had 1.14 times higher risk of developing coronary heart disease than healthy people (11). Also, this study showed that the prevalence of coronary heart disease in cross-sectional studies was significantly greater (1.59 times) in people with periodontal disease than those without the periodontal disease (11).

A respiratory infection can be developed by bacteria that can infect the lower respiratory tract during inhalation of infectious aerosols and the spread of infection from contiguous and/or extrapulmonary sites. Saliva and dental plaque in a patient with periodontal disease have the pathogens including *A. actinomycetemcomitans*, *Actinomyces israelii*, *Capnocytophaga spp*, *Chlamydia pneumonia*, *E. corrodens*, *F. nucleatum*, *Fusobacterium necrophorum*, *P. gingivalis*, *P. intermedia* and *Streptococcus constellatus* that spread to the lower airways (12-14). A previous study showed that respiratory pathogens isolated from dental plaque and bronchoalveolar lavage fluid from the patients were genetically the same, and the study concluded dental plaque could serve as a reservoir for respiratory pathogens (15). One study revealed patients with periodontitis had 3 times more risk of developing nosocomial pneumonia when compared with patients without periodontitis (16).

Several studies showed an association between periodontal pathogen and oral, pancreatic, head and neck, and lung cancers (17-20). The pathogen *P. gingivalis* was significantly elevated in oral squamous and esophagus squamous cell carcinoma patients when compared with healthy mucosa (17,19). Another study in mice showed the periodontal pathogens *P. gingivalis* and *F. nucleatum* stimulated tumorigenesis by interaction with oral epithelial cells, and it is mediated by the host innate immune system

(21). Also, colorectal carcinoma is associated with the excessive abundance of *F. nucleatum* in the intestinal microbiota of colorectal carcinoma patients. It is considered that oral *F. nucleatum* could migrate to the intestinal tract and could cause deleterious inflammatory infections (22), and *F. nucleatum* was significantly observed in colonic adenomas relative to surrounding tissues (23). Also, the pathogen was identified in stool samples from patients with colorectal carcinoma when compared with patients without colorectal carcinoma (23).

Diabetes mellitus and periodontitis present a two-way association in which one affects the other. A chronic infection caused by periodontitis can lead to exacerbated inflammatory responses which would result in reduced metabolic control of blood glucose level and increased insulin requirements (24). Previous studies showed that patients with acute bacterial infection demonstrated severe and long-lasting insulin resistance (25), and periodontal treatment improved glycemic control in type 2 diabetic patients (26). Conversely, diabetic patients had a 3-fold increase in the risk of periodontitis when compared with non-diabetic patients, and periodontitis was found in 58% of type 1 diabetes patients and 15% of non-diabetic people (27,28).

Alzheimer's disease which is a progressive neurodegenerative disease is bi-directionally associated with periodontitis, and an oral-health study showed patients with brain injury had a higher prevalence of poor oral health parameters and chronic periodontitis (29,30). An increase of pro-inflammatory cytokines was detected in elderly patients with Alzheimer's disease and periodontitis (31), and the treatment of the inflammation could protect the brain from further damage and decrease the rate of Alzheimer's disease progression (32,33).

Pregnancy is associated with gingivitis and periodontitis because of hormonal changes, and approximately 40% of pregnant women have clinical evidence of periodontal disease (34). There are two hypotheses about the association between oral disease and adverse pregnancy outcomes: 1) translocation of oral pathogens from the affected oral cavity to the placenta and reaching the intra-amniotic fluid and fetal circulation and 2) the systemic dissemination of endotoxins or inflammatory mediators derived from a periodontal disease that could affect the development of the fetus or spontaneous miscarriage (35,36). In

rodent studies, the lipopolysaccharide from periodontal pathogens *P. gingivalis* induced placental and fetal growth restriction and resorption, and antibodies produced against *P. gingivalis* that were passively administered caused fetal loss (37,38).

1.3.2. Oral disease and quality of life

Oral disease strongly impacts the quality of life, and the quality of life associated with oral disease is called “Oral Health-Related Quality of Life (OHRQoL)”. One of the problems related to oral disease is dental pain from which 66% of patients with the oral disease suffer (39). In the United Kingdom, more than 90% of dental caries in pre-school children are not treated (40), and this condition affects the nutritional status, growth and well-being of the children (41-43). The previous study indicated that 80% of 3.2 year-olds with dental caries weighed 8.7% less than their ideal weight, compared with only 1.7% of those without dental caries (15.2 kg vs. 16.2 kg) because of not only decrease of food intake but also disturbed sleep, decrease of growth hormones and increase of metabolic rate during infection (41). Studies assessed OHRQoL of children with dental caries and their parents; the studies revealed that dental caries decrease their OHRQoL including school learning, sleep, playing, food intake (i.e. feeling pain when they eat something hot, cold and sweet), family work and family finance when compared with those without dental caries. They additionally showed that the treatment improves the OHRQoL (44-46). Periodontal disease is associated with pain, psychological discomfort and food intake (47-49), and is also related to mental health problems including anxiety, melancholy and suicide thought (47). The basic concept of dental disease management is considered as maintenance of the OHRQoL. It focuses on prevention of the disease including education about proper oral hygiene, dietary modification with respect to the use of sugar and sticky food and a healthy diet, and only minimal possible invasive procedure should be performed (6).

2. Dental disease and its impact on cats

2.1. Periodontal disease

Periodontal disease is one of the most common diseases in small animals (7) as well as humans. One study involved 31,484 dogs and 15,226 cats from 52 private veterinary clinics in the United States showed dental calculus and gingivitis were the most commonly reported disorders, and the prevalence was approximately 22% and 16%, respectively (7). Also, the other study indicated that 96% of cats in a colony had periodontal inflammation (50), and a radiographic study found 72% of 147 cats had some degree of periodontitis (51). The etiology of periodontal disease in small animals is similar to humans. Feline periodontal disease is also caused by the host's inflammation against plaque and is described in 2 stages: gingivitis and periodontitis. Plaque is consisted of more than 300 bacterial species (56% of aerobic bacteria and 44% of anaerobic bacteria) in cats (52), and the deposition of the tooth surface occurs within hours of the teeth erupting or being cleaned (53). When the layer of plaque is mineralized by saliva and gingival crevicular fluid, calculus is created, and it starts within hours of plaque accumulation, and the process may be complete within 2 weeks (53). Although calculus itself is not the direct cause of periodontal disease, it makes the surface of teeth rough and which accelerates the accumulation of pathogenic plaque bacteria.

Gingivitis is an initial and reversible condition, and the inflammation is limited in the gingiva. It can be reversed with dental prophylaxis including dental scaling and/or daily home care including tooth brushing, specific diet and chemical supplements (i.e. chlorhexidine, soluble zinc salts, xylitol-containing water and powdered algal food supplements) (53). Gingivitis, however, would progress to periodontitis if the condition is not treated. Periodontitis is characterized as the destruction of attachment structures including cementum, alveolar bone and periodontal ligament. Periodontal management is consisted of preventive and treatment procedures (52). Preventive procedures include dental scaling and polishing, and the procedures remove the cause of the disease and allow the tissues to restore themselves to health. Also, dental scaling provides an accurate dental examination to evaluate if the healthy attachment exists (52). Treatment procedures include the correction of existing loss of attachment or tooth extraction. The

choice of the treatments depends on the preference of the owners, extent and health of the gingiva surrounding the tooth, extent of loss of attachment, mobility of the tooth, and furcation exposure (loss of alveolar bone between the roots of multi-rooted teeth) (52).

2.2. Oral health and systemic diseases in cats

In dogs, the association between periodontal and systemic diseases has been reported (55,56). A historical cohort observational study involved 59,296 dogs showed the risks of dilated cardiomyopathy, hypertrophic cardiomyopathy, endocarditis and mitral valve insufficiency were significantly higher in dogs with periodontal disease when compared with those without the periodontal disease (55). The same authors studied the association between periodontal disease and chronic kidney disease in 164,706 dogs with periodontal disease, and the risk of chronic kidney disease based on blood concentration of creatinine was significantly higher in dogs with the periodontal disease when compared with those without the periodontal disease (56). In cats, however, less information is available. One study investigated the risk factors of chronic kidney disease in 1,230 cats showed that cats with periodontal disease had 1.82 times more risk of development of chronic kidney disease than those without the periodontal disease (57). Also, the association between periodontal disease and diabetes mellitus has been reported in one study and one case report (58,59). In the study, the prevalence of periodontal disease was significantly higher in diabetic Burmese cats (49%) when compared with non-diabetic Burmese cats (21%) (59). Also, the case report concluded that the treatment of periodontal disease improved glycemic control, which would be due to the improvement of insulin resistance from periodontal inflammation (58). However, to the author's knowledge, this latter study has never been published in full.

2.3. Feline chronic gingivostomatitis

Feline chronic gingivostomatitis (FCGS) is a painful condition and characterized by protracted oral inflammation that crosses the mucogingival junction and extends to the buccal and caudal oral mucosa (60). The prevalence of FCGS is reported as 0.7 to 12%

in Europe (61,62). A previous retrospective case-control study showed all cats with FCGS had periodontitis, and 77% of the cats had an alveolar bone loss (63). The prevalence was significantly higher when compared with cats without FCGS (63). The other common clinical signs are dysphagia, halitosis, sialorrhea, weight loss, intense oral discomfort, oral hemorrhage, lackluster and fragile coat, and the prevalence of these signs were reported as 88.2, 76.5, 47.1, 41.2, 35.3, 17.6, and 11.8%, respectively (64). FCGS is the results of bacterial and viral infection including feline calicivirus, feline leukemia virus, feline immunodeficiency virus, feline herpesvirus, *Bartonella henselae*, and *Pasteurella multocida* (64-69). FCGS are thought to be the result of an abnormal inflammatory immune response and the subsequent release of reactive oxygen species from inflammatory cells in the gingiva (70,71). Lesions are primarily infiltrated by lymphocytes and plasma cells, neutrophils, macrophage-like cells, and mast cells (72). A previous study of immunohistochemistry revealed that feline leukemia virus antigens were detected in the epithelium and the inflammatory infiltrate from 30.8% of the cats with FCGS, but feline calicivirus antigens were not detected in the lesions (64). The authors concluded that feline calicivirus would play an important role in oral inflammation in early FCGS, but it does not induce persistent infection (64). The presence of dental plaque is considered to be a major contributing factor of FCGS (73), and minimizing oral bacteria by mechanical removal is important to reduce oral inflammation (70). The gold standard method to treat FCGS is surgical interventions including full-mouth or near full-mouth (premolar and molar) tooth extraction (65,74-78). The complete and partial remission rates have been reported as 67 to 80% (65,74,78). In one study involving cats with stomatitis, a good outcome was observed in cats with the improvement of abnormal behaviors including vocalization, hiding, lethargy, halitosis, decreased grooming, bruxism or oral discharge at first postoperative recheck examination (78). Also, one case report showed that the use of CO_2 laser as adjunctive treatment after tooth extractions improved oral inflammation (76). If the surgical intervention does not resolve the problem, medical intervention by immunosuppressive therapy including cyclosporine is performed (79,80). A retrospective study investigating the efficacy of cyclosporine in cats with FCGS that did not previously undergo tooth extraction showed 50% of cats went into remission, and the remaining cats had a fair to good improvement (79). Also, a randomized, placebo-controlled, double-

blinded clinical study involved cats that were previously received tooth extractions showed that 45.5% of cats achieved clinical remission, and 77.8% of cats showed a > 40.0% improvement based on a semi-quantitative stomatitis score (80). Several studies reported the efficacy of the other treatments including fresh mesenchymal stem cells (MSCs) (autologous and allogeneic) (81,82), recombinant feline interferon omega (83-85), bovine lactoferrin (86) and thalidomide with lactoferrin (87). Of the cats treated with autologous MSCs, 71.4% (5/7) of them responded to the treatment [complete clinical remission (n = 3) and substantial clinical improvement (n = 2)] (81), while 57% (4/7) of cats treated with allogeneic MSCs responded to the treatment [complete clinical remission (n = 2) and substantial clinical improvement (n = 2)] (82). Two studies investigated the efficacy of recombinant feline interferon omega and bovine lactoferrin revealed that clinical improvement was observed in 45% and 77% of cats, respectively (84,86). Although it was tested only one cat, the administration of thalidomide with lactoferrin succeeded in treating FCGS (87).

2.4. Feline orofacial pain syndrome

Feline orofacial pain syndrome (FOPS) is characterized by behavioral signs associated with severe oral discomfort including excessive licking and chewing movements, pawing at the mouth and face and tongue mutilation (86,87). FOSP is considered a neuropathic pain disorder and is considered to be related to the hereditary tendency (88,89). A retrospective study involved 113 cats with FOPS showed that 89% (101/113) of cats were Burmese or Burmese cross (88). In the study, the presence of periodontal disease and environmental stress (living in a multi-cat household, following the introduction of a new kitten, the death of their primary caregiver and moving to a new house) were considered as the triggers of discomfort (88). Also, an eruption of a permanent tooth is considered as the trigger, and approximately 17% of cats had the first FOPS event less than 6 months old in the previous study (88,89). The authors of the study hypothesized that Burmese might have dysfunction of central and/or ganglion processing of trigeminal sensory information, and clinical signs of FOPS seem to begin when the endings of the trigeminal nerves are damaged or sensitized by tooth eruption or oral inflammation (88).

Management of FOPS is consisted of treatment of periodontal disease, pharmacological interventions and stress reduction (improvement of environment and/or use of commercially available feline facial pheromone F3) (88,89). Tooth extractions for the treatment of periodontal disease improved signs of FOPS in 66% (35/53) of cats. Clinical signs of 1/53 cat, however, was worse than before the tooth extractions, and 2/53 cats got the clinical signs of FOPS immediately after the extractions (88). This phenomenon could be caused by inappropriate perioperative pain management (88,89). The efficacy of pharmacological treatments including non-steroidal anti-inflammatory drugs (NSAIDs: meloxicam, ketoprofen or carprofen), corticosteroids (prednisolone, methylprednisolone or dexamethasone), antibiotics, combination anti-inflammatory and antibiotic treatment, opioids (buprenorphine, pethidine or butorphanol), anti-epileptic drugs (phenobarbital or diazepam) and amitriptyline were reported, and the success rates were 39, 65, 25, 43, 50, 94 and 56%, respectively (88). Also, in the study, gabapentin and carbamazepine were used in single cases, and they were reported as being effective for alleviating oral pain (88).

3. Specific dental nociception pathways

Noxious input is transmitted to the brain through pain pathways including transduction (the conversion of noxious stimuli into an action potential), transmission (the propagation of the action potential by primary afferent neurons to the dorsal horn of the spinal cord), modulation [up/down regulation of signals by neurotransmitters including glutamate, substance P (SP), γ-aminobutyric acid (GABA) and glycine], projection (the conveyance of nociceptive information through the spinal cord to the brain) and perception (the integration of the nociceptive information by the brain) (90). Dental pain is delivered by the trigeminal nervous system, and which has a unique structure and functions for processing orofacial nociception as well as non-noxious sensations when compared with the spinal nervous system (91).

The orofacial area is mainly innervated by three main branches of the trigeminal nerve (i.e. ophthalmic, maxillary, and mandibular nerves). Oral structures are innervated by small diameter Aδ-fibers (the rapid, acute, sharp pain) and unmyelinated C-fibers (the

delayed, more diffuse, dull pain) that process orofacial nociception, and they receive various stimuli including thermal (hot/cold), mechanical, or chemical stimulation (91). Hot and cold stimulation at trigeminal primary afferent neurons are conveyed via transient receptor potential (TRP) ion channel family: vanilloid 1 and 2 (TRPV1, TRPV2) and TRP M member 8 (TRPM8) and TRP ankyrin 1 (TRPA1), respectively (92).

Sensory signals from the teeth are transmitted by dental nerves that innervate the tooth pulp and dentin (91). The signals from dental nerves are delivered to the trigeminal ganglion (TG), and the information at the trigeminal nerve is then conveyed from trigeminal afferents via various neurotransmitters such as glutamate and SP to second-order neurons in the trigeminal sensory nuclear complex (TSNC) in the brainstem and the upper cervical (C1-C2) spinal cord that is the primary sites of synaptic integration for sensory inputs from the face and oral cavity via TG. These neurotransmitters bind to such as α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors and neurokinin (NK) 1 receptors. Several inflammatory mediators and growth factors lead to sprouting and changes in neuropeptide expression of dental afferent neurons, and the changes cause increased pain sensitivity (93). TSNC is consisted of the principal sensory nucleus (PrV) and the trigeminal spinal nucleus (SpV), and they convey non-noxious and noxious sensory information, respectively. SpV is also subdivided into three nuclei: oralis (Vo), interpolaris (Vi), and caudalis (Vc). Vc has a similar laminar organization to the spinal dorsal horn (laminar I–II), and it is considered as the critical region of the projection of nociceptive information to the posterior ventromedial thalamus (VPM) that goes to somatosensory and medial thalamic nuclei that goes to limbic cortices. The somatosensory-VPM pathway and the limbic cortices-medial thalamic nuclei pathway are known to be involved in the sensory-discriminative aspect of pain and the motivational and affective part of the pain, respectively (94). The pain signals can be modulated by descending pain pathways (inhibitory or facilitatory) by acting on Vc and C1-C2 nociceptive neurons via periaqueductal gray (PAG) and ventromedial medulla (RVM).

Increased SP production is related to inflammatory dental pain, and concentration of SP in inflamed teeth and irreversible pulpitis were 100 times and 1000 times higher,

respectively, when compared with a normal dental condition (95). SP exerts the effect via NK 1, and the activation enhances the activity of TRPV1 and purinergic P2X3 receptors and may sensitize peripheral sensory neurons (96). Also, SP can activate the production of pro-inflammatory mediators and cytokines via leukocytes and may induce the release of histamine that increases vascular permeability causing pulsating inflammatory pain via mast cells (95). Some neuropeptides including calcitonin gene-related peptide (CGRP) and chemical mediators including serotonin (5-HT), and cytokines are considered to be associated with inflammatory dental pain. A study in experimentally induced dental caries in ferrets, c-Fos expression in Vc was significantly fewer in ferrets treated with CGRP antibodies when compared with a control group (97). A study in humans, 5-HT enhances capsaicin-evoked CGRP release from trigeminal nociceptors (98). TRPV1 ion channel is one of the specific transducers of nociception, and it was upregulated in TG following experimentally induced pulpitis in mice (99). In another study in rats, teeth pulp inflammation enhanced the activity of TG neurons innervating adjacent non-inflamed teeth and TRPV1 expression in TG, resulting in the ectopic persistent tooth-pulp pain following pulp inflammation of adjacent teeth (100). Also, TRPV1 induces the release of SP, CGRP, and it plays a role in pain detection and tissue inflammation (92). These facts show increased expression of TRPV1 in dental primary afferent neurons contributes to dental hypersensitivity.

In healthy teeth, noxious thermal stimuli usually do not elicit pain because of the enamel. However, once the layer is damaged and the dentin is exposed, slight thermal stimuli evoke dental pain. Also, different from the skin, exposed dentin feels pain against the weak air-puff stimulus (101), and the fact shows that the teeth have a specific nociceptive mechanism by which they detect nociceptive stimulation in inflammatory conditions or when dentin is exposed. Also, dental pain would be caused by additional hypersensitivity mechanisms. The mechanism of activation of pulp nerves are hypothesized as three theories including the neural theory, the hydrodynamic theory and the odontoblastic transduction theory (92,101), and the activation of dental primary afferent nerves delivers dental nociception to central nervous system in each theory.

The neural theory is a theory that the pulpal nerve endings are directly activated by external stimulation, and the transduction of a specific stimulus to an electrical nerve impulse is mediated by nociceptive receptors expressed in dental primary afferent neurons (101). The majority of the dental primary afferent neurons involve the TRP ion channel family: TRPV1 and TRPV2 are warm-sensitive receptors, and TRPA1 and TRPM8 are cold/mechanosensitive and cold-sensitive ion channel receptors. TRPA1 and TRPM8 are co-expressed with TRPV1-positive dental afferent neurons, and which would be the reason why it is difficult to discriminate between hot and cold stimuli applied to teeth (101).

The hydrodynamic theory is a theory that tooth pain is induced by hydrostatic pressure applied to inflamed pulp tissue encased within hard dentin structures (102). This theory would be supported by the fact that sudden and intense tooth pain is caused by innocuous stimuli including water spray, air-puff or sweet substances. Pain induced by chronic pulpits is characterized as “pulsating pain”, and the generation of dental pain might involve the detection of mechanical forces. The cause of dental pain related to mechanical forces is generated by the movement (i.e. inward and outward) of dentinal fluid. Inward and outward movements of the fluid are caused by hot and cold stimuli, respectively. Outward movement is faster than inward movement by hot stimuli, and family the cold sensation is more readily detected by Aδ fibers as a sharp pain in early pulpits. As pulpitis progresses, C-fibers are sensitized and activated by the inward movement of dentinal fluid by hot stimuli, and it is perceived as a dull pain. In the TRP ion channel family, TRPV1, TRPV2 and TRPA1 are mainly considered as mechanosensitive receptors that are related to the hydrodynamic theory. TRPA1 is related to both cold hyperalgesia and mechano-sensation, and the dual functions would be able to explain why dental pain elicited by a light air-puff is sometimes confused with cold nociception (101).

The odontoblastic transduction theory is a theory that odontoblasts that constitute a cell layer at the outermost part of the dental pulp and secrete mineralized calcium matrix to form dentin act as sensory transducers of noxious stimuli into electrical signals transmitted to neighboring nerve endings (101). Several TRP ion channel family including TRPV1, TRPV2, TRPV4, TRPA1 and TRPM8 are expressed on the odontoblastic membrane, and

they enable odontoblasts to detect fluid movement (i.e. external stimuli) within the dental tubules and play an important role in the transduction of heat and cold stimulation and dental pain.

4. Pain assessment and dental pain-induced behaviors

Pain is the 4th vital sign to be monitored following temperature and rates of pulse and respiration (103), and assessment of pain is important to make a decision if additional analgesics should be administered. In veterinary medicine, however, it is sometimes difficult to evaluate, since the patients could not tell their levels of pain. As a result, the provision of appropriate analgesics is challenging in veterinary medicine (104), especially in cats because of their unique character (105). Even in veterinary teaching hospitals, less than 50% of veterinarians considered the patients undergoing castration or ovariohysterectomy were adequately treated with analgesics according to studies performed in 2002 and 2003 (106,107). By the early 2000s, this percentage in general practice was worse, and it was less than 40% and 30% in cats undergoing ovariohysterectomy and castration, respectively (108-110). After the late 2000s to the 2010s, the proportion improved and more than 80% of the veterinarians considered their patients received adequate analgesic therapy (111-113). According to these studies, cats were less likely to receive analgesics when compared with dogs (109-113). Although the use of analgesics for routine surgeries has increased, the confidence of knowledge about assessment and management of pain is still low (112), and pain assessment tools were routinely used by only 17% (113).

Historically, unidimensional scales including simple descriptive scales, visual analog scale, numerical rating scales, dynamic interactive visual analog scale have been used for acute pain assessment in veterinary medicine (114). These scales are simple, but highly subjective, and they do not demonstrate sensitivity in detecting small changes in pain intensity. Also, the inter-rater reliability of the unidimensional scales is variable when several veterinarians scored pain after surgery in dogs (115,116).

Recently, knowledge of small animal pain assessment has dramatically developed. Some multidimensional composite pain scales which include interactive, physiological and behavioral items are currently available for acute pain assessment in dogs and cats in addition to the unidimensional scales (117-122). When developing new pain scales, the scales should be assessed for validity (i.e. if the instrument is measuring what it is intended to measure), reliability (i.e. if the scale produces consistent results when repeated over time or between different raters), and responsiveness (i.e. if the scale has an ability to detect clinically important changes including worsening pain or improvement after analgesic intervention) (114). In feline medicine, only two multidimensional pain scales: the Glasgow Composite Measure Pain Scale-Feline (CMPS-F) and UNESP-Botucatu Multidimensional Composite Pain Scale (MCPS), and one facial expression-based pain scale: the Feline Grimace Scale (FGS) are considered as validated scales and have been evaluated intervention level (i.e. the analgesic threshold) (118,119,123). Colorado State University Feline Acute Pain Scale (CSU-FAPS) which is pain scoring system with moderate to good inter-rater reliability, and further validation is still required in cats (122). Briefly, CMPS-F and MCPS include several questions about behaviors including vocalization, activity/posture, attention to wound, response to palpation, response to touch and demeanor. The latest version of CMPS-F also includes facial expression, and the inclusion of the facial expression improved the prevalence of misclassification from 26.7% to 17.6% when compared with the previous version (121,124). The MCPS include physiological variables (appetite and blood pressure) in addition to the above domains, and the variables could be omitted without compromising the pain assessment (119). The FGS is consisted of 5 action units including ears, eyes, muzzle, whisker and head position, and the change of the facial expression is evaluated (123).

In addition to these pain scales, international veterinary experts in feline medicine identified 25 core signs of pain (sufficient to indicate pain when they occur) associated with several conditions including orthopedics, cancer, urinary tract, pancreatitis, ophthalmic, dental/oral, general trauma and surgical pain (125). In the study, however, only one specific behavior associated with dental pain was identified (i.e. change in the form of feeding behavior). Although some pain scales have been used to evaluate dental

pain in cats, the validation of these scales for dental pain is not confirmed yet (8,126). A scale is currently developed for the purpose of oral and maxillofacial pain assessment in dogs and cats after medical or surgical intervention (127). The construct validity, criterion validity and internal consistency of the scale were confirmed (127). In other species, some specific behaviors have been identified. In Malayan sun bears with dental pain, behaviors including general activity, social behaviors, stereotypes, eating-related and orofacial behaviors were evaluated (128). The bears that received dental treatment took significantly longer to eat soft porridge and hard sugarcane preoperatively when compared with postoperative 4 weeks (128). A similar finding was observed in a rodent experimental study (129). In this study, rats undergoing surgery of pulp exposure had significantly increased duration of food intake up to postoperative 8 days when compared with control rats. In cats, although the total amount of dry food intake during a 6-hour period was not significantly different between before and after dental treatment, dental treatment had a significant effect on the time to ingest food, whereby cats ate more quickly after treatment when compared with before treatment (130). Overall, although dental pain-induced behaviors in some species have been reported, the knowledge of dental pain is still anecdotal and there is a lack of strong evidence-based information.

5. Pain management strategies in small animals

Provision of appropriate management and prevention of perioperative pain is essential to improve the quality of life and animal welfare, and which may influence postoperative patients' outcomes (131). Optimal pain relief is achieved with the use of different classes of analgesics including opioids, local anesthetics, NSAIDs, and other adjuvant analgesics [i.e. α-2 adrenergic receptor agonists, NMDA receptor antagonists and gabapentin], and this strategy is well known as multimodal analgesia (103). Multimodal analgesia is the combination of 2 or more drugs allows decreasing the dosage and adverse effects of each drug by acting at different pain pathways (103,132,133). Although several analgesics are available for perioperative dental pain management in small animal practice, and some literature reviews have been reported, few reports regarding the analgesic efficacy of each drug have been studied in patients with dental disease in veterinary medicine.

5.1. Opioids

Opioids are drugs that have opiate-like activities and play an important role in perioperative pain management as a part of multimodal analgesia in veterinary medicine (103,133). Opioid receptors are mainly classified as three groups: μ, δ and ƙ. Opioids decrease the release of excitatory neurotransmitters, resulting in decreased transmission within the spinal cord (135). Full μ-opioid agonists including morphine, hydromorphone, fentanyl, remifentanil and methadone produce the most profound analgesic effects. Also, partial μ-opioid agonist (buprenorphine), ƙ agonist/μ antagonist (butorphanol) and opioid-like drug (tramadol) are available in veterinary medicine. Opioids are commonly administered intravenously (IV), intramuscularly (IM), or subcutaneously (SC), or oral transmucossaly (OTM) in the clinical setting. Previous studies indicate that IV or IM routes should be chosen for rapid onset and for maximum analgesic effect even the SC route produces less pain during administration when compared with the IM route (136-139). For the purpose of this literature review, a brief summary of opioid analgesics will be introduced to the reader.

Morphine (suggested dose, dosing frequency and routes of administration in cats; 0.2-0.4 mg/kg, q 4-6h, IM/IV) is a full agonist at opioid receptors (103,134,135). Although morphine is one of the most commonly used opioids, it is not licensed for use in veterinary species. The production of the metabolite morphine-6-glucuronide that is considered to be responsible for part of the analgesic effects of morphine is limited in cats (140); this limitation may affect the analgesic efficacy of morphine in cats. Systemic administration of morphine significantly increased thermal nociceptive threshold (at lateral thorax) from 4 to 6 hours (141) and reduced volatile anesthetic requirement by 28 ± 9% and 12 ± 4% when morphine was administered at 1 mg/kg and 0.1 mg/kg IV, respectively (142). The antinociceptive effect against electrical stimulation on the tooth pulp was evaluated in dogs, and IV and intrathecal administration of morphine 0.1 mg/kg significantly increased the threshold when compared with the control group (143). Morphine is relatively hydrophilic, and long-lasting analgesia can be achieved via epidural administration

(141,142). The main adverse effects after IV/IM/SC injection of morphine are vomiting and histamine release (103).

Hydromorphone (0.025-0.1 mg/kg, q 4–6h, IM/IV) is a semi-synthetic full μ-opioid agonist analgesic, and its duration of effect is similar to morphine (103,134,135), but histamine release is less likely to occur when compared with morphine (146). Another consideration of the administration of hydromorphone in cats would be hyperthermia even other opioids including morphine, buprenorphine and butorphanol could also induce hyperthermia (147-149). A retrospective study indicated 64-69% of cats developed hyperthermia (defined as rectal temperature > 40 ℃) (147). The antinociceptive effect of hydromorphone is dose-dependent, and IV administration of hydromorphone at 0.1 mg/kg increased the thermal nociceptive threshold up to 200 minutes when compared with baseline (150). Also, hydromorphone (0.1 mg/kg IV) produced inhalant anesthesia sparing effect (approximately 28%) when compared with the control group (151).

Fentanyl [Bolus 1-10 μg/kg IV + constant rate infusion (CRI) 2-15 μg/kg/h, patch 25 μg/h] is a potent short-acting, lipid-soluble, synthetic μ-opioid agonist (103,134,135). Since fentanyl is rapidly distributed and eliminated after IV injection, CRI should be chosen for perioperative analgesia and reduction of inhalant anesthetic requirements (152). A single bolus of fentanyl at 10 μg/kg significantly increased the thermal nociceptive threshold up to 2.5 hours when compared with the control group (153). Also, another study showed that a fentanyl infusion of 5 μg/kg/h following a bolus of 5 μg/kg produced thermal and mechanical antinociception (154). According to these 2 studies, the plasma concentration of fentanyl > 1.07 and > 1.3 ng/mL were necessary for antinociception, respectively (153,154). For long-term analgesia, the analgesic efficacy of transdermal administration of fentanyl (fentanyl patch) has been studied. In general, fentanyl patches have a long onset period, and it is recommended to place at least 12 hours before analgesia in cats (103). In a randomized controlled clinical trial in cats undergoing onychectomy and/or ovariohysterectomy or castration, cats that received a fentanyl patch (25 μg/h 4 hours before the surgery) had significantly lower postoperative pain scores throughout the study (i.e. up to postoperative 40 hours) when compared with the cats receiving butorphanol (0.5 mg/kg IM as premedication followed by another 0.2 mg/kg at

extubation) (155). An experimental study showed that fentanyl patches (25 μg/h) reduced inhalant anesthesia requirements against mechanical stimulation by approximately 18% (156). Since there is great individual variability in drug absorption and analgesic efficacy, close pain assessment is essential when a fentanyl patch is applied, and additional analgesics should be administered appropriately if needed.

Remifentanil (4-60 μg/kg/h CRI) is a potent μ-opioid agonist, and it has a more rapid onset of action and shorter context-sensitive half-life (time required for the plasma concentration to decrease by 50% after the termination of an infusion) after prolonged infusion when compared with fentanyl (103,134,135,157). Remifentanil is metabolized by non-specific plasma and tissue esterases (158), and this pathway of extra-hepatic metabolism has an advantage in patients with hepatic or renal disease. When compared with baseline, remifentanil CRI at 0.25, 0.5 and 1 μg/kg/min decreased isoflurane requirements during electrical nociceptive stimulation by 23.4 ± 7.9, 29.8 ± 8.3 and 26 ± 9.4%, respectively, and there were no significant differences between dosages, which indicates there would be a ceiling effect (157).

Methadone (0.3-0.6 mg/kg, q 4h, IM/IV) is a synthetic μ-opioid agonist, and this drug works as an NMDA receptor antagonist and plays an important role in the descending pain pathways by inhibiting the reuptake of serotonin and noradrenaline and by blocking the nicotinic cholinergic receptors (103,134,135). Experimental studies in cats indicated methadone 0.3 mg/kg IV increased mechanical nociceptive thresholds up to 4 hours after administration (159), and decreased sevoflurane requirements by 25% (159).

Buprenorphine [0.02-0.04 mg/kg (formulation of 0.3 mg/mL), q 4-8h, SC/IM/IV/OTM and 0.24 mg/kg (formulation of 1.8 mg/mL), q 24h, SC] is a highly lipophilic semi-synthetic partial μ agonist (103,135,136,161-163). The antinociceptive effects and duration depend on dosage regimens and individual variability (139,164-167). Buprenorphine 0.01-0.02 mg/kg IM or IV increased the thermal nociceptive threshold up to post-administration 12 hours (141,164). Another study showed that a higher dose (0.02 and 0.04 mg/kg IV) produced better mechanical antinociception and increased the duration of action when compared with 0.01 mg/kg (166). The volatile anesthetic sparing effects of buprenorphine are controversial, and a study showed IV administration of buprenorphine at 0.005 and

0.05 mg/kg did not produce a clinically relevant isoflurane sparing effect (11-17 %) (142). A high concentrated formulation of buprenorphine (Simbadol, 1.8 mg/mL) is currently available in the United States, and SC administration of Simbadol at 0.24 mg/kg produced a thermal antinociceptive effect up to 30 hours post-administration in cats (162). OTM administration of buprenorphine is also reported in some studies, and the bioavailability is considered approximately 23-32 % (162,168). OTM administration of buprenorphine at 0.02 mg/kg produced a similar thermal antinociceptive effect to IV administration (up to 6 hours after administration) (164). On the other hand, in a clinical study in cats undergoing ovariohysterectomy, postoperative pain scores in the OTM route were significantly higher up to postoperative 12 hours when compared with IM and IV groups (167). In a clinical study in cats with gingivostomatitis, although OTM administration of buprenorphine produced an analgesic effect and higher prevalence of soft food intake when compared with the control group, the bioavailability (19.5%) was lower than cats without gingivostomatitis (169).

Butorphanol (0.2–0.4 mg/kg, q 1–2h, IM/IV) is a synthetic opioid with ƙ agonist//μ antagonist effects (103,134,135). Butorphanol increased thermal nociceptive threshold up to approximately 3 hours, decreased pain scores after ovariohysterectomy during postoperative 2 hours when compared with the control group (170,171), and also produces a mild volatile anesthetic sparing effect (18-51%) (142,151). When combined with dexmedetomidine IM, cats undergoing various surgeries and receiving butorphanol had higher postoperative pain scores than the ones receiving buprenorphine (172), but was also associated with a lower prevalence of vomiting and a superior depth of sedation and anesthesia (173,174). Tramadol [2-4 mg/kg, q 6–8h, IM/IV/ Orally (PO)] produce the analgesic effect by binding to the μ-opioid receptors and by interfering with the neuronal release and reuptake of serotonin and noradrenaline in the descending inhibitory pathways (103,134,135). PO administration of tramadol produced dose-dependent thermal antinociceptive effects up to 6 hours and a volatile anesthetic sparing effect of 40% (151,175).

5.2. Local anesthetics

Local anesthesia techniques are the only way to provide complete analgesia/antinociception with minimal adverse effects (133,176). Local anesthetics prevent cell membrane depolarization of the afferent neuron. The permeability of the neuronal cell membrane to sodium ions is decreased which prevents the nerve impulse beyond the area of the block (133,176). The use of local anesthetics contributes to not only the relief of perioperative pain but also decreases the amount of systemic administration of other analgesics including opioids and decrease of inhalant anesthetics (177,178). Duration of blockade (i.e. uptake into the membrane) depends on the local anesthetic itself, and their concentration and volumes of administration. Commonly used local anesthetics in veterinary medicine include lidocaine, mepivacaine, bupivacaine and ropivacaine (103). Toxicity including central nervous systems and cardiovascular system depressions result from high plasma concentrations of local anesthetics (179).

Lidocaine (maximum recommended doses in dogs and cats: 8 and 6 mg/kg, respectively) has fast onset (5-10 minutes) and intermediate potency (2: when the potency of procaine is defined as one) and duration (90-200 minutes), and the minimal recommended concentration is reported as 0.125% (103). Although systemic administration of lidocaine can be performed in some species for the purpose of analgesia and inhalant anesthetic sparing effects, it is not recommended in cats because of cardiovascular depression, which is greater than an equipotent minimum alveolar concentration of volatile anesthetics (180). Mepivacaine (maximum recommended doses of dogs/cats: 4.5 and 3 mg/kg, respectively) has similar onset (3-10 minutes), relative potency (2) and duration (120-240 minutes) to lidocaine (103). Bupivacaine (maximum recommended doses of 2 mg/kg in dogs and cats) has intermediate onset (10-20 minutes) and relative high potency (8) and long duration (180-600 minutes), and the minimal recommended concentration is reported as 0.25% (103). Ropivacaine (maximum recommended doses in dogs and cats: 3 and 1.5 mg/kg, respectively) has similar onset (15-20 minutes) to bupivacaine but lower relative potency (4) and duration (90-360 minutes) (103). In a rodent study, ropivacaine had delayed cardiotoxic and neurotoxic side effects and a wider margin of safety when compared with bupivacaine (181).

The local anesthetic techniques are strongly recommended when oral surgical or periodontal procedures are performed (177). The most common sites for dental nerve blocks include the infraorbital foramen, the caudal maxillary nerve, the middle mental foramen, and the mandibular foramen (103). Although infraorbital and mental nerve blocks are commonly used for rostral to middle dental surgery (103), in dogs, mental nerve block did not reliably provide the desensitization against mechanical stimulation to the rostral area; success rates of sensory blockade in incisor to molar teeth were less than 50% (182). A similar result of desensitization at premolar and molar teeth were observed in another study in dogs (183). In cats, success rates of infraorbital and inferior alveolar nerves were lower when compared with dogs and this was attributed to lack of obvious anatomical landmarks for these techniques in cats (184). In canine cadaver studies, the success rate of the maxillary block with the percutaneous approach varies between 21.6 and 82.3% (185,186). For improvement of the success rates, novel techniques including the infraorbital approach by using an intravenous catheter and transorbital approach were investigated, and the success rates were 64.9 and 88.2%, respectively (185,186).

Only a few studies about the analgesic efficacy and inhalant anesthetic sparing effects of dental nerve blocks have been reported in dogs and cats. In cats undergoing one or more tooth extractions, cats premedicated with acepromazine, buprenorphine and medetomidine intramuscularly and administered maxillary and inferior alveolar nerve blocks with lidocaine 2% and bupivacaine 0.25% had lower postoperative pain scores when compared with those without dental nerve blocks (8). In a study in dogs, infraorbital block with mepivacaine 2% provided dental antinociceptive effect against electrical stimulation and reduced isoflurane requirements up to 23% when compared with the control group (9).

The addition of some drugs including epinephrine, α-2 adrenergic receptor agonists and opioids to local anesthetics to improve the duration of dental nerve blocks has been amply reported in human medicine, but only one study has been reported in dogs (187-192). The addition of epinephrine causes vasoconstriction, and which is considered to extend the duration of dental blocks (187,188). Similar to the effect of epinephrine, the addition of dexmedetomidine to local anesthetics improved postoperative pain scores and reduced

the prevalence of rescue analgesia in humans undergoing cleft palate repair (190). Also, one study showed that the onset of action was faster, and the duration of analgesia was longer in the lidocaine and dexmedetomidine group when compared with the lidocaine and epinephrine group (192). In human and dog studies, the addition of buprenorphine extended the duration of bupivacaine's analgesic effect up to post-administration 28.18 and 96 hours, respectively (189,191).

Some complications related to dental nerve blocks have been reported in dogs and cats. In a case report in a cat with mandibular squamous cell carcinoma undergoing mandibulectomy, the cat developed severe cardiovascular depression requiring cardiopulmonary resuscitation following a mandibular nerve block with bupivacaine (193). The authors concluded the execution of peripheral nerve blocks close to a neoplastic process where the local inflammatory response and neovascularization exist might cause a rapid uptake of the local anesthetic. Also, retrobulbar hematoma, ophthalmologic complications including globe penetration transient and/or permanent vision loss, buphthalmos, intraocular hypertension, a mature cataract and posterior synechiae that required medical and/or surgical interventions have been reported (194-197).

5.3. Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs are one of the most commonly used drugs for perioperative dental pain management in combination with opioids and local anesthetics (103,198). Since most of the NSAIDs are available orally and are not controlled drugs like opioids, it is easy to prescribe them as "take-home analgesics". Analgesic and adverse effects are produced by the inhibition of cyclooxygenase (COX) and the reduction of prostaglandin (PG) production. There are at least two distinct forms of COX; COX-1 is responsible for basal PG production for normal homeostasis including gastroprotection, reproduction, wound healing, bone metabolism, nerve development and growth, and immune responses, whereas COX-2 is primarily associated with the inflammatory effects of PG. In kidneys, both COX-1 and 2 are expressed and play an important role in the maintenance of renal perfusion and autoregulation (103). Consequently, the inhibition of COX-2 activity specifically is the therapeutic target of NSAIDs, and COX-2 selective NSAIDs are

commonly used for the treatment of inflammation in veterinary medicine (103). NSAIDs are widely used for 3-7 days depending on how severe oral pain is or how invasive surgical procedures are (103,178). Several studies showed long-term NSAIDs therapy could be safe and efficacious when administered to geriatric cats with osteoarthritis for 6 months when administered relatively low doses (198). NSAID-associated renal adverse effects were not detected even in cats with IRIS-stage I and II chronic kidney disease when meloxicam or robenacoxib were administered for > 6 months and 28 days, respectively (299,200). Also, a study in cats undergoing dental surgery reported that pre-anesthesia administration of meloxicam and carprofen did not cause significant alterations in renal function (i.e. glomerular filtration rate and urinary N-acetyl-β-D-glucosaminidase activity) 24 hours after general anesthesia and dental surgery (201). Although the administration of NSAIDs for small animals with most types of oral pain is recommended in review articles and specific guidelines (178,202,203), the perioperative analgesic efficacy has not been scientifically investigated in cats. As described above, the use of NSAIDs for the treatment of FOPS has been studied, and the success rate was low (39%) (88).

5.4. Adjuvant analgesics

The administration of adjuvant analgesics including α-2 adrenergic receptor agonists, NMDA receptor antagonists and gabapentin are recommended for management of oral pain in review articles and specific guidelines (178,202,203).

α-2 adrenergic receptor agonists including medetomidine (6-20 μg/kg IM/IV) and dexmedetomidine (1-10 μg/kg IM/IV, 10-20 μg/kg OTM for cats) produce sedation, analgesia and muscle relaxation by binding to α-2 adrenergic receptors in the dorsal horn of the spinal cord, cerebral cortex and locus coeruleus (103,178). Common side effects include cardiovascular depression, hypothermia, decreases in sympathetic tone and gastrointestinal motility, increases in urinary output, and hyperglycemia (103). One of the benefits is that α-2 adrenergic receptor agonists are reversible by antagonists (i.e. atipamezole). Sedative and antinociceptive effects of medetomidine (80 μg/kg IM) and dexmedetomidine (40 μg/kg IM) were evaluated in cats, and the study showed that both drugs produced significant sedative and antinociceptive effects up to post-administration

180 and 120 minutes, respectively, when compared with baseline (204). In the study, the most commonly observed adverse event was vomiting (7%). An isoflurane sparing effect has been reported in cats, and the study showed that dexmedetomidine at 3 μg/kg/h decreased isoflurane requirements from 1.83% to 0.82% when compared with a control group (205). α-2 adrenergic receptor agonists are commonly administered in combination with opioids, acepromazine and/or benzodiazepines.

Ketamine (2-5 mg/kg IM/IV for induction, 2-20 μg/kg/min as infusion for antihyperalgesia) produces an analgesic effect by antagonizing NMDA receptors which are activated by glycine and glutamate (neurotransmitters) during sustained nociception in the dorsal horn of the spinal cord (103). Due to the effects on transmission and modulation of nociceptive stimuli, infusion of ketamine has an important role in the effect of preventing/resetting central sensitization and wind-up (206). Ketamine also has volatile anesthetic sparing effects when administered in high doses. A study showed that the cats administered ketamine at 23, 46 and 115 μg/kg/min reduced isoflurane requirements by 45, 63 and 75%, respectively, when compared with baseline (207).

Gabapentin (5-30 mg/kg PO) is a structural analogue of GABA, but does not interact with GABA receptors to produce analgesia (103,208). Although the mechanism of gabapentin is not fully understood, it is considered that the analgesic effect is produced by inhibition of N-type voltage-dependent neuronal calcium channels which reduces calcium influx into neurons and the release of excitatory and inhibitory neurotransmitters, altering channel trafficking and stimulating the movement of channels away from neuronal cell membranes (103,209). The postoperative analgesic efficacy of gabapentin was studied in cats undergoing ovariohysterectomy (210). In the study, the cats administered gabapentin (17.3 ± 3.7 mg/kg PO) in combination with buprenorphine at 0.02 mg/kg IM had significantly lower prevalence of rescue analgesia based on CMPS-F when compared with the cats administered buprenorphine alone (210). On the other hand, thermal antinociceptive effect and an isoflurane sparing effect were not observed when gabapentin was administered at 5, 10 and 30 mg/kg PO and intravenously to achieve target plasma concentrations up to 16 ng/mL, respectively (211,212). Therefore, gabapentin should be administered as a part of multimodal analgesic protocols (213,214).

6. Objectives and hypotheses

As described above, there is little availability of information about the specific clinical signs associated with oral pain in cats, and there is a need to investigate them to improve the feline health and welfare.

The objectives of this thesis were: 1) to identify the specific behaviors associated with oral disease by using video assessment, and to correlate them with the real-time pain scores, 2) to assess the impact of oral disease and pain on food intake and feeding-related behaviors, 3) to determine the effects of oral disease treatment on behavior, pain scores and food intake, 4) to assess the inter-rater reliability of the FGS in cats undergoing dental extractions and 5) to evaluate the analgesic efficacy and adverse events of a high-concentration formulation of buprenorphine hydrochloride formulation (Simbadol, 1.8 mg/mL) in comparison with a standard buprenorphine hydrochloride formulation (Vetergesic, 0.3 mg/mL) as part of a multimodal regimen in cats undergoing dental extractions. The hypotheses were: 1) specific behaviors associated with oral disease would be identified and correlated with real-time pain scores, 2) cats with severe oral disease would have lower food intake and higher pain scores and require rescue analgesia when compared with cats with no/minimal oral disease, 3) treatment of oral disease would reduce the prevalence of specific behaviors and pain scores and improve food consumption of these animals, 4) the FGS scores scored by different raters would be reliable in cats undergoing dental extractions and 5) both Simbadol and Vetergesic would produce similar postoperative pain scores, adverse events and timing and prevalence of rescue analgesia when using CMPS-F.

Article 1: A multidisciplinary study of pain in cats undergoing dental extractions: A prospective, blinded, clinical trial

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Funding acquisition: PS.

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Abstract

This study aimed to evaluate pain scores, analgesic requirements, food intake and serum inflammatory cytokines in cats before and after clinically recommended dental treatment. Twenty-four cats were included in a prospective, blinded clinical trial. Cats were equally divided into minimal (minimal dental treatment) or severe (multiple dental extractions) oral disease groups. They were admitted (day 0) and underwent oral examination/radiographs/ treatment under general anesthesia (day 1; acepromazine-hydromorphone-propofol-isoflurane-meloxicam-local anesthetic blocks). Serum inflammatory cytokines were measured on days 0 and 6. Pain was scored using the Glasgow composite measure pain scale-feline (CMPS-F). Rescue analgesia was administered with hydromorphone if CMPS-F ≥ 5/20. Dry and soft food intake (%) during 3 minutes and 2 hours, and daily soft food were calculated. The Cochran-Mantel-Haenszel and Chi-square tests, Spearman's rank correlation and linear mixed models were used for statistical analysis (alpha = 0.05). Pain scores were significantly increased in cats with severe disease when compared with baseline (up to day 4) and minimal disease (all postoperative time points). Prevalence of rescue analgesia was significantly higher in severe (91.7%) than minimal disease (0%); analgesics were required up to day 3. Pain scores and frequency of rescue analgesia were significantly correlated with the number of tooth extractions, gingival and calculus index. Prevalence of rescue analgesia was significantly correlated with the number of missing teeth, teeth extractions and gingival index. Dry and soft food intake during 3 minutes, and dry food intake during 2 hours were significantly lower in the severe than minimal disease group throughout the study. Some cytokines differed between groups between day 0 and day 6 and were associated with the presence of tooth resorption and number of missing tooth and tooth fractures. Long-term analgesia is required after dental extractions in cats with severe oral disease. This condition reduces food intake and influences serum inflammatory cytokines.

Introduction

Pain is a serious welfare issue that produces long-term distress with significant deleterious effects affecting quality of life (QoL) in humans (42,49,215,216). Periodontal disease including gingivitis and periodontitis, is one of the most commonly reported diseases in humans and companion animals (7,41,42,52,217). In cats, it produces pain, inflammation, dysphagia, halitosis, weight loss and oral hemorrhage; aggressive full-mouth extractions are commonly required as treatment (218,219). Nevertheless, pain scores and analgesic requirements have not been systematically investigated in cats with oral disease undergoing dental extractions. It is unknown how oral treatment can affect soft and dry food intake perioperatively which could significantly impact the nutritional status of these patients.

Gram-negative bacteria are the major pathogen of periodontal disease. They release endotoxins (lipopolysaccharides; LPS) that mediate the release of inflammatory cytokines [e.g. interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)] which are strongly correlated with the progression of the disease in humans (220-223). In veterinary medicine, local inflammatory cytokines have been evaluated in dental resorptive lesion and bone in cats with periodontal disease (224-226). However, serum concentrations of inflammatory cytokines in cats with periodontal disease have not been studied. The study of serum inflammatory cytokines could provide valuable insight in the pathogenesis of oral disease in cats.

The objectives of this study were to evaluate the effects of oral disease and its treatment on pain scores, analgesic requirements, food intake and serum inflammatory cytokines in cats with severe or minimal oral disease. The hypotheses were that cats with severe disease would have higher pain scores and analgesic requirements and reduced food intake than those with minimal disease before and after dental extractions, and that concentrations of serum inflammatory cytokines would differ between groups.

Materials and methods

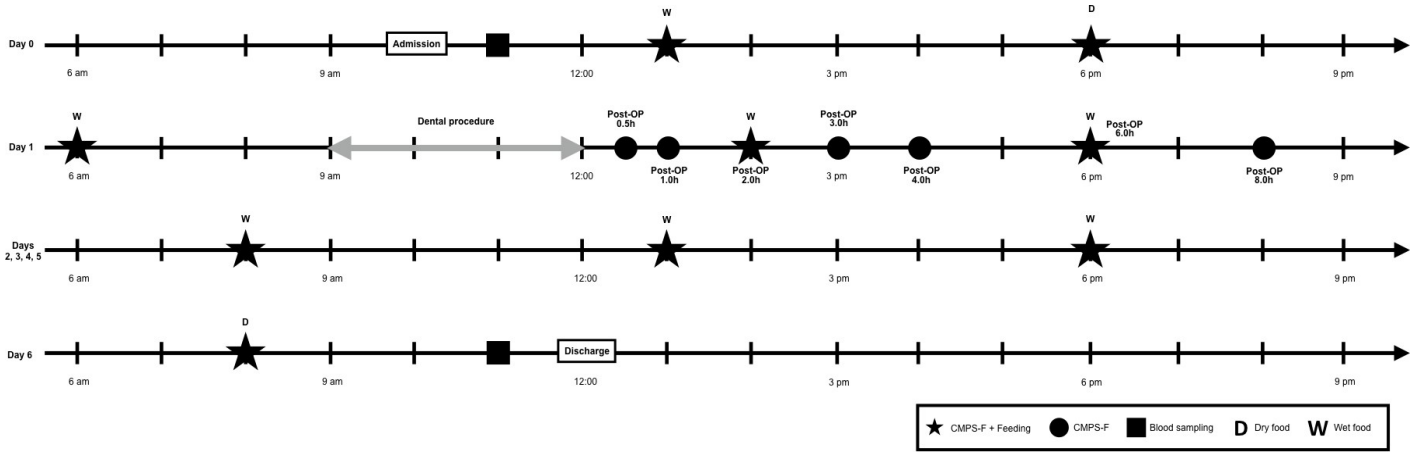
Study design

This study was approved by the Institutional Animal Care and Use Committee of the Université de Montréal (protocol 17-Rech-1890) and is reported according to the CONSORT guidelines (227). The experimental study was performed at the Centre hospitalier universitaire vétérinaire (CHUV), the veterinary teaching hospital of the Faculty of Veterinary Medicine of the Université de Montréal, between July 2017 and February 2018. The study design was a prospective, blinded, controlled clinical trial. Randomization was not feasible because cats were allocated to one of two groups according to their disease severity (severely versus minimally affected cats).

Animals

Twenty-four adult (> 1 year of age) cats of different breeds and gender with naturally-occurring oral disease were studied. Cats were recruited by the investigators (PS and BM) from shelter facilities after informed written consent based on the severity of oral disease. Initial oral examination was performed by the local shelter veterinarians who were aware of the presence or absence of clinical signs related to dental disease. They were admitted approximately 24 hours before general anesthesia (day 0) for dental treatment which was performed on day 1. Cats were discharged on day 6 (7 days after arrival and 6 days after treatment of oral disease) (Figure 1). Animals were housed in stainless steel cages in a cat only ward with access to water ad libitum, toys, litter box and bedding. A cardboard box was also provided offering additional shelter and an elevated surface. Environmental enrichment was provided following the guidelines of the American Association of Feline Practitioners (228).

Figure 1. Schematic of time points for assessment of pain and food intake in cats undergoing oral treatment for 7 days. CMPS-F: Glasgow Composite Measure Pain Scale-Feline



Inclusion and exclusion criteria

Cats with body condition score between 4-6 out of 9, and with minimal or severe oral disease were included (229). Inclusion was also based on history, medical records, complete physical examination, and hematology and biochemical panel. Feral cats were excluded. Cats were also excluded if they had concurrent medical conditions, systemic disorders (e.g. cancer, renal, cardiovascular, hepatic, or gastrointestinal disease) and/or received any medication including analgesics and antibiotics for up to 10 days before the study had begun.

Treatment of oral disease

Group allocation

Complete dental examination and radiography were performed, and patients underwent dental cleaning and dental extractions (if needed) by a board-certified dentist (YD) and a resident (CP) of the American Veterinary Dental College (AVDC). Staging of periodontal disease was based on dental examination, radiography, gingival index, calculus index, number of teeth resorbed, and fractured and/or missing (230-232). Group allocation (minimal or severe) was determined upon recruitment after oral examination and

confirmed according to a scoring system based on the type and number of extracted teeth: canine tooth - 3 points; third premolar of maxilla or molar of mandible - 2 points, second premolar of maxilla or premolar of mandible - 1 point. A score of 2 points was given if seven or more incisive teeth and/or first premolars of the mandible were extracted; otherwise a score of 1 point was given if six or fewer teeth were removed. The total dental score was calculated, and cats were allocated to the minimal disease group if dental score ≤ 7, and to the severe disease group if dental score was ≥ 8. This cut-off was determined based on the expected level of pain that would be clinically significant in cats with score ≥ 8.

Anesthesia and analgesic protocol

All cats were premedicated with an intramuscular (IM) injection of acepromazine (0.02 mg/kg; Acepromazine maleate, Gentès & Bolduc, Saint-Hyacinthe, QC, Canada) and hydromorphone (0.1 mg/kg; Hydromorphone hydrochloride 2 mg/kg, Sandoz, Boucherville, QC, Canada). A eutectic mixture of local anesthetic cream (EMLA cream lidocaine 2.5% and procaine 2.5% cream, Astra Zeneca, Mississauga, ON, Canada) was applied to the skin over the cephalic vein after clipping and covered with plastic film and adhesive bandage. Approximately 20 minutes later, a 22-G intravenous (IV) catheter was aseptically placed in the cephalic vein and induction of anesthesia was performed with propofol (Propoflo 28, Zoetis, Kirkland, QC, Canada) administered IV to effect (4.0 ± 1.2 mg/kg). After spraying the arytenoid cartilages with 0.05 mL of lidocaine 2% (Lidocaine hydrochloride sterile injection, 20 mg/mL, Vétoquinol N.-A.Inc, Lavaltrie, QC, Canada), cats were intubated with an appropriately sized cuffed endotracheal tube and connected to a coaxial Mapleson D system. Anesthesia was maintained with isoflurane (Isoflurane USP, Fresenius Kabi, Toronto, ON, Canada) carried in 100% oxygen. Monitoring was performed with a multiparametric monitor (Lifewindow 6000V Veterinary Multiparameter Monitor; Digicare Animal Health, Boynton Beach, FL, USA) including pulse oximetry, electrocardiography, capnography, inspired and expired concentrations of isoflurane, indirect blood pressure via oscillometry, and rectal temperature probe. Blood pressure was also monitored with a Doppler flow monitor and a sphygmomanometer (233,234). The cuff width used for blood pressure monitoring was approximately 40% of the limb circumference. A balanced crystalloid solution was administered (2-5 mL/kg/h) based on

patient needs throughout the anesthetic period. Cats received local anesthetic blocks with bupivacaine 0.5% (Sensorcaine, AstraZeneca, ON, Canada) using a 25-G needle based on the anatomical sites of dental extraction(s) including the mental, infraorbital, maxillary and/or inferior alveolar mandibular nerve blocks approximately 20 minutes before teeth extraction. The total dose of bupivacaine for all anesthetic blocks was up to 2 mg/kg. Meloxicam (0.2 mg/kg, subcutaneously, Metacam 5 mg/mL Solution for Injection; Boehringer Ingelheim, Burlington, ON, Canada) was administered at the end of the surgical procedure. Three additional doses of meloxicam (0.05 mg/kg, Metacam 0.5 mg/mL Oral Suspension for Cats; Boehringer Ingelheim, Burlington, ON, Canada) were administered orally at 24, 48 and 72 hours after the first dose according to label recommendations in Canada.

Pain assessment

Pain assessment was performed by a trained observer [RW] who was blinded to the disease severity using the Glasgow composite measure pain scale-feline (CMPS-F) (121). Pain was evaluated preoperatively (at 1 pm and 6 pm on day 0, approximately three hours before the dental procedure on day 1, and at 0.5, 1, 2, 3, 4, 6, 8 hours after the end of anesthesia on day 1. Pain was also assessed at 8 am, 1 pm and 6 pm on days 2, 3, 4, 5 and again at 8 am on day 6 according to Figure 1. Baseline pain scores were calculated using the mean of three preoperative values. Rescue analgesia was administered if CMPS-F scores were ≥ 5/20 with hydromorphone either at 0.05 mg/kg IV (if the intravenous catheter was in place, first 24 hours after surgery) or 0.1 mg/kg IM (if the intravenous catheter had been removed). In this case, pain was reassessed 30 minutes later to ensure the patient's comfort. Based on previous literature on the duration of hydromorphone in cats, pain scores obtained within 2 hours of IV and within 6 hours of IM injection of rescue analgesia were excluded from statistical analysis. (235,236). However, pain assessment was performed systematically until the end of the study period.

Food intake

All cats were fed a commercially available dry food (Hill's Science Diet, Adult Optimal Care – Dry; Hill's Pet Nutrition Canada Inc., Mississauga, ON, Canada) on days 0 and 6. A commercial canned prescription recovery diet (Hill's Prescription Diet a/d; Hill's Pet Nutrition Canada Inc., Mississauga, ON, Canada) was provided at all other time points during the study (Figure 1). Total amount (100%) of food offered per day was calculated based on the following equation (kcal): 70 × body weight (kg)$^{0.75}$ (237). Cats were served 33.3% of their daily total amount at each time point (Figure 1). Food intake (percentage of the total amount offered) during 3 minutes and 2 hours was calculated (except on the morning of day 1 when cats were fed for only 3 minutes) for each time point; any remaining food was removed after 2 hours. Daily food intake (percentage of the total amount offered) was calculated using the mean of three meals offered per day. Baseline food intake (%) was calculated using the mean of the two preoperative soft food meals. Food intake obtained within 2 hours of IV and within 6 hours of IM injection of rescue analgesia were excluded from statistical analysis, but assessments were continued until the end of the study.

Inflammatory cytokines

Sample collection

Whole blood was collected via venipuncture using the jugular vein on days 0 (before the first pain and food intake assessments) and 6 (after final pain and food intake assessments) (Figure 1), placed into a sterile 3 mL anticoagulant-free glass tube (Monoject Blood Collection Tube; Covidien Canada, Saint-Laurent, QC, Canada) and allowed to clot at room temperature for 40 minutes. Clotted samples were then centrifuged at 3000 rpm for 10 minutes, and serum was removed, aliquoted, and stored in cryovials at -80℃ until final analyses (238).

Evaluation of serum concentration

Samples were warmed to room temperature and analyzed for concentrations of 19 analytes using commercially available feline-specific multiplex cytokine kits (FCYTMAG-20K-PMX, Luminex Corporation, TX, USA). The kit was used according to the manufacturer's recommendations. The plate was analyzed using a dedicated reader (MAGPIX, Luminex Corporation, TX, USA) and software (xPONENT v.4.2, Luminex Corporation, TX, USA). The quality control samples, standard curves, and bead counts were assessed and conformed to manufacturer recommendations. Analytes with concentrations more than 50% out of the range of analysis were excluded from the analyses.

Statistical analyses

Statistical analyses were performed using standard statistical software (SAS version 9.3; SAS Institute, Cary, NC, USA). Power analysis revealed that this study needed 8 cats per group to detect a difference of three points in the CMPS-F pain scores between the two groups 80% of the time using an alpha value of 0.05, and a standard deviation within group of 2 points. These values were based on clinical experience where changes in three points in CMPS-F were clinically relevant. Therefore, the authors decided to include 12 cats per group to assure adequate power considering the individual variability of oral disease in cats. Data were tested for normality using a Shapiro-Wilk test. Demographic data for each treatment group were compared using two-sample t-tests or Mann-Whitney U tests where appropriate. The CMPS-F ordinal scores were compared between baseline and each time point, and between dental severity groups at each time point using Cochran-Mantel-Haenszel statistics. Prevalence of rescue analgesia between groups was compared using the exact chi-square test. Serum concentration of inflammatory cytokines were compared after $\log_{10}$ transformation between days 0 and 6, and between dental severity groups using a linear mixed model followed by contrasts between pairs of means using the Benjamini-Hochberg sequential alpha adjustment procedure. Food intake was compared with baseline and between dental severity groups using a linear mixed model with the same contrast comparisons. Correlation between pain scores and the frequency

and prevalence of rescue analgesia, and periodontal staging, gingival index, calculus index, number of tooth resorption, tooth fracture and missing were evaluated using Spearman's correlation. The alpha level was set at 5% throughout.

Results

Descriptive statistics for age, body weight, body condition score, surgery (time elapsed from the first scaling until the end of scaling or placement of the last suture) and anesthesia (time elapsed from induction of propofol to turning off the vaporizer dial) times, and dental score and number of extracted teeth are presented in Table 1. Cats in the minimal disease group were typically younger and lighter and required less time for surgery and anesthesia than those in the severe group (Table 1). One cat from the minimal disease group was excluded because of wound dehiscence in the postoperative period requiring further treatment. Therefore, only preoperative data of this cat was included in the analysis.

Table 1. Demographic data, surgery and anesthesia times in cats with minimal and severe oral disease.

Variable	Minimal (n = 12)	Severe (n = 12)	p value
Age (years)	3.6 (2.0)	8.5 (2.2)	< 0.0001
Body weight (kg)	4.0 (0.6)	5.8 (1.9)	0.007
Body condition score (1-9)	5 (5-6)	6 (4-6)	0.078
Surgery time (minutes)	98.8 (47.4)	261.0 (72.2)	< 0.0001
Anesthesia time (minutes)	103.8 (48.2)	274.7 (70.3)	< 0.0001
Dental score	1 (0-4)	17 (8-28)	< 0.0001
Number of extracted teeth	2 (0-5)	17 (8-30)	< 0.0001

Values are expressed as mean (SD) with exception of body condition score, dental score and number of extracted teeth which are reported as median (min-max).

Pain assessment

Pain scores in each group are shown in Table 2. In the severe group, CMPS-F scores were significantly higher at 1, 2, 3, 4, 6 and 8 hours on day 1, at all three time points on day 2 and at 1 pm on days 3 and 4 when compared with baseline. In the minimal group, there were no significant differences between baseline and any postoperative time point ($p = 0.13$). CMPS-F scores in the severe group were significantly higher than the minimal group at all postoperative time points. Rescue analgesia was administered to 11 cats in the severe group (91.7%) and to none in the minimal group (0%) ($p < 0.0001$) (Table 3). CMPS-F scores and the frequency of rescue analgesia were significantly correlated with number of tooth extractions ($r = 0.84$, $p = 0.0001$ and $r = 0.83$, $p = < 0.0001$; respectively), gingival index ($r = 0.70$, $p = 0.001$ and $r = 0.67$, $p = 0.003$; respectively) and calculus index ($r = 0.48$, $p = 0.02$, and $r = 0.47$, $p = 0.03$; respectively). Prevalence of rescue analgesia was significantly correlated with the number of missing teeth and tooth extractions, gingival index and calculus index ($r = 0.46$, $p = 0.03$; $r = 0.78$, $p = < 0.0001$; $r = 0.72$, $p = < 0.0001$ and $r = 0.56$, $p = 0.006$; respectively).

Table 2. Median (min-max) of pain scores using the Glasgow Composite Pain Scale Feline (CMPS-F) in cats with minimal or severe oral disease undergoing dental extractions throughout the study.

Time point		Minimal CMPS-F scores	Severe CMPS-F scores	p value comparisons with baseline (severe group only)	p value between groups
Baseline		0 (0)	0 (0-2)		0.083
Day 1 (Postoperative)	0.5 hours	0 (0)	0.5 (0-4)	0.12	0.020
	1 hours	0 (0)	1 (0-6)	0.042	0.017
	2 hours	0 (0)	2 (0-5)	0.016	0.0006
	3 hours	0 (0-1)	3 (1-7)	0.016	0.0011
	4 hours	0 (0-1)	5 (0-7)	0.029	0.0011
	6 hours	0 (0)	2 (1-6)	0.039	0.0013
	8 hours	0 (0)	1.5 (1-5)	0.027	0.0015
Day 2	8 am	0 (0-1)	2 (0-5)	0.008	0.0005
	1 pm	0 (0-1)	2 (1-6)	0.013	0.0008
	6 pm	0 (0-3)	2 (0-7)	0.039	0.0150
Day 3	8 am	0 (0)	1 (0-5)	0.053	0.0038
	1 pm	0 (0)	2 (0-2)	0.016	0.0001
	6 pm	0 (0)	1 (0-6)	0.052	0.0056
Day 4	8 am	0 (0)	1 (0-4)	0.096	0.0036
	1 pm	0 (0)	1.5 (0.4)	0.043	0.0028
	6 pm	0 (0)	1 (0-3)	0.061	0.0009
Day 5	8 am	0 (0)	1 (0-2)	0.083	0.0026
	1 pm	0 (0)	0.5 (0-1)	0.74	0.0076
	6 pm	0 (0)	0.5 (0-2)	0.32	0.013
Day 6		0 (0)	1 (0-2)	0.25	0.0062

Table 3. Number of cats receiving rescue analgesia at each time point during the study.

Group	Day 1 (postoperative)							Day 2	Day 3	Days 4, 5, 6	Total	p value
	0.5 h	1 h	2 h	3 h	4 h	6 h	8 h					
Minimal	0	0	0	0	0	0	0	0	0	0	0 (0%)	< 0.0001
Severe	0	2	1	2	5	2	2	5	2	0	21 (91.7%)	

Food intake

Soft food intake

In the severe group, soft food intake during 3 minutes and daily soft food intake during 3 minutes were significantly lower than the minimal group throughout the study (Table 4). When compared with baseline, food intake was significantly higher in the minimal group at 6 hours after the end of anesthesia and significantly lower in the severe group in the morning of day 4. Soft food intake during 2 hours and daily soft food intake during 2 hours were not significantly different between groups.

Dry food intake

Dry food intake during 3 minutes and 2 hours was significantly lower in the severe group when compared with the minimal group at days 0 and 6 (Table 4).

Table 4. Mean (SD) of dry and soft food intake (%) in cats with minimal or severe oral disease undergoing dental treatment.

Time point		Food intake (%) during 3 minutes			Food intake (%) during 2 hours			p value compared with baseline (3 minutes)		p value compared with baseline (2 hours)	
		Minimal	Severe	p value between groups	Minimal	Severe	p value between groups	Minimal	Severe	Minimal	Severe
Baseline	Soft food	63.7 (9.0)	42.4 (7.9)	0.0538	94.3 (4.8)	85.1 (9.2)	0.44				
	Dry food	77.1 (6.0)	28.1 (6.1)	0.0001	94.9 (5.1)	63.6 (11.5)	0.012				
	2 hours	67.4 (12.3)	29.2 (8.3)	0.0002	87.3 (9.40)	86.1 (10.5)	0.99	0.26	0.062	0.45	0.86
Day 1 (Postoperative) Soft food	6 hours	83.6 (7.8)	33.2 (10.4)	< 0.0001	100 (0.0)	72.7 (18.9)	0.002*	0.001	0.028*	0.45	0.025*
	Daily food intake	75.5 (9.5)	27.7 (27.7)	< 0.0001	93.6 (4.7)	52.1 (48.0)	0.003*	0.020*	0.049*	0.95	0.23
Day 2	8 am	59.7 (9.5)	35.4 (7.1)	0.0102*	90.9 (9.1)	75.5 (11.6)	0.092	0.80	0.17	0.77	0.14
Soft food	1 pm	66.2 (8.0)	40.1 (8.0)	0.0097*	93.8 (4.2)	77.2 (10.1)	0.11	0.62	0.52	0.94	0.24
	6 pm	66.9 (8.8)	37.1 (3.5)	0.0041*	92.4 (7.6)	85.2 (6.6)	0.25	0.46	0.40	0.99	0.61
	Daily food intake	64.3 (7.8)	36.0 (5.1)	0.004	92.4 (5.5)	72.6 (7.2)	0.020*	0.75	0.90	0.68	0.78
Day 3	8 am	60.7 (8.8)	29.8 (5.0)	0.0057	93.4 (6.6)	89.4 (5.2)	0.35	0.65	0.057	0.94	0.87
Soft food	1 pm	68.3 (7.6)	31.6 (5.0)	0.0017*	100 (0.0)	74.2 (11.0)	0.0155*	0.40	0.21	0.94	0.17
	6 pm	76.1 (9.5)	33.1 (4.0)	0.0001	90.9 (9.1)	82.2 (9.0)	0.28	0.052	0.19	0.99	0.47
	Daily food intake	68.4 (7.9)	31.5 (4.0)	0.0007	94.8 (5.2)	82.0 (5.1)	0.020*	0.79	0.67	0.73	0.24
Day 4	8 am	57.5 (9.6)	16.7 (5.0)	0.0004	91.9 (8.1)	81.0 (9.2)	0.16	0.39	0.0003	0.95	0.38
Soft food	1 pm	70.0 (6.8)	38.7 (5.7)	0.0129*	100 (0)	94.8 (5.3)	0.63	0.41	0.91	0.45	0.26
	6 pm	74.0 (8.2)	22.2 (4.5)	< 0.0001	91.9 (8.1)	73.6 (9.3)	0.044*	0.094	0.0076*	0.95	0.10
	Daily food intake	67.2 (7.4)	25.9 (4.2)	0.0002	94.6 (5.4)	83.1 (5.8)	0.045*	0.98	0.17	0.75	0.09
Day 5	8 am	70.4 (9.2)	22.3 (5.4)	< 0.0001	93.4 (6.6)	81.8 (10.5)	0.25	0.27	0.004*	0.94	0.68
Soft food	1 pm	73.2 (8.7)	38.0 (3.7)	0.0025*	100 (0.0)	92.0 (5.8)	0.43	0.097	0.86	0.45	0.45
	6 pm	75.4 (9.0)	29.9 (4.7)	0.0002	91.9 (8.1)	78.9 (9.9)	0.19	0.09	0.18	0.95	0.43
	Daily food intake	73.0 (8.4)	30.0 (3.9)	< 0.0001	95.1 (4.9)	84.3 (7.5)	0.16	0.27	0.54	0.70	0.007*
Day 6 Dry food	8 am	71.2 (8.2)	18.6 (5.5)	< 0.0001	91.9 (8.1)	57.7 (11.4)	0.009	0.48	0.19	0.84	0.68

* not significant after adjustment.

Inflammatory cytokines

GM-CSF was excluded from statistical analyses because more than 50% of concentrations were beyond the reference range. Interferon (IFN)-γ, IL-4, IL-6, IL-8, regulated on activation-normal T cell expressed and secreted (RANTES), stem cell factor (SCF), and monocyte chemoattractant protein (MCP) -1 were significantly lower on day 6 than on day 0 in cats with severe oral disease (Table 5). The concentrations of SCF were

significantly higher in cats with severe than those with minimal disease on day 0. IL-12p40 was significantly higher on day 6 than on day 0 in both groups. There were positive associations between soluble FAS (sFAS), IL-6, stromal cell-derived factor (SDF) -1, and MCP-1, and the number of teeth resorption (p = 0.048, 0.028, 0.012, and 0.047, respectively), between sFAS and the number of missing teeth (p = 0.02), between keratinocyte chemoattractant (KC) and the number of teeth fracture (p = 0.038), and a negative association between sFAS and TNF-α and the number of teeth fracture (p = 0.03 and 0.011, respectively).

Table 5. Log_{10} transformed least squares means (SEM) for serum concentrations of inflammatory cytokines and chemokines in cats with minimal or severe oral disease undergoing dental treatment.

Analyte	Minimal			Severe				
	Day 0	Day 6	p value between days 0 and 6	Day 0	Day 6	p value between days 0 and 6	p value between groups at day 0	p value between groups at day 6
sFAS	0.82 (0.17)	0.74 (0.17)	0.33	0.78 (0.17)	0.66 (0.17)	0.12	0.87	0.75
FLT-3L	1.79 (0.06)	1.81 (0.06)	0.52	1.79 (0.06)	1.79 (0.06)	0.95	0.93	0.76
IFN-γ	1.89 (0.14)	1.87 (0.14)	0.63	2.28 (0.14)	2.16 (0.14)	0.008	0.07	0.17
IL-1β	1.17 (0.32)	1.21 (0.32)	0.66	1.56 (0.25)	1.39 (0.25)	0.022*	0.37	0.68
IL-2	1.21 (0.22)	1.03 (0.22)	0.08	1.47 (0.22)	1.41 (0.22)	0.53	0.43	0.24
IL-4	2.04 (0.17)	1.99 (0.17)	0.28	2.57 (0.17)	2.41 (0.17)	0.0004	0.04*	0.10
IL-6	1.95 (0.15)	1.91 (0.15)	0.28	2.07 (0.15)	1.93 (0.15)	0.0006	0.94	0.94
IL-8	1.39 (0.09)	1.24 (0.09)	0.003	1.59 (0.09)	1.46 (0.09)	0.006	0.11	0.08
IL-12p40	2.52 (0.08)	2.62 (0.08)	0.005	2.52 (0.08)	2.66 (0.08)	0.0002	0.99	0.72
IL-13	1.35 (0.15)	1.28 (0.15)	0.11	1.34 (0.14)	1.26 (0.14)	0.03*	0.97	0.90
IL-18	2.00 (0.10)	2.02 (0.11)	0.71	2.12 (0.10)	1.99 (0.10)	0.031*	0.41	0.81
KC	0.68 (0.32)	0.89 (0.33)	0.49	1.02 (0.28)	1.58 (0.26)	0.033*	0.44	0.13
MCP-1	2.89 (0.13)	2.82 (0.13)	0.11	3.07 (0.13)	2.97 (0.13)	0.007	0.48	0.48
PDGF-BB	2.76 (0.13)	2.77 (0.13)	0.93	2.98 (0.14)	2.75 (0.14)	0.015*	0.28	0.94
RANTES	1.51 (0.07)	1.48 (0.07)	0.44	1.54 (0.07)	1.43 (0.07)	0.003	0.75	0.58
SCF	2.07 (0.07)	2.05 (0.07)	0.69	2.35 (0.07)	2.22 (0.07)	0.0003	0.10	0.006
SDF-1	2.86 (0.09)	2.91 (0.09)	0.39	2.91 (0.10)	2.84 (0.10)	0.85	0.77	0.61
TNF-α	1.40 (0.45)	1.50 (0.45)	0.57	2.24 (0.39)	1.97 (0.39)	0.11	0.21	0.48

* not significant after adjustment.

Discussion

This study showed that cats with severe oral disease undergoing dental treatment had significantly higher postoperative pain scores and analgesic requirements, and significantly lower soft and dry food intake when compared with those with minimal oral disease. Additionally, pain scores and frequency and prevalence of rescue analgesia were correlated with some dental parameters and specific serum inflammatory cytokines.

Postoperative pain scores in the severe group were significantly higher than baseline up to day 4 and throughout the study when compared with the minimal disease group. These findings suggest that not only individuals with severe oral disease have discomfort and pain before surgery but also that multiple dental extractions produce severe pain despite the administration of multimodal analgesia including local anesthetic blocks, a non-steroidal anti-inflammatory drug and an opioid. This study also suggests that cats should be hospitalized after multiple dental extractions for appropriate pain management. These patients require the administration of opioids for pain relief up to 72 hours after surgery based on the high prevalence of postoperative rescue analgesia in the severe group (91.7%). Their pain scores are still higher than those cats with minimal oral disease up to 6 days after surgery. There is a clear need for long-term analgesia and better analgesic treatments to address these patients' needs. Pain scores and frequency and prevalence of rescue analgesia were correlated with specific dental parameters (i.e. number of tooth extractions, gingival and calculus index). Based on these findings, postoperative analgesic requirements might be predicted based on intraoperative oral examination and number of extractions and missing teeth. Oral disease has been considered a welfare issue in the guidelines of the World Small Animal Association Dental Standardization Committee with a negative impact in the quality of life of companion animals (178). Indeed, it produces severe pain and inflammation before and after treatment in cats (240). Future studies are warranted to determine oral disease pain-induced behaviors that would facilitate feline pain recognition and assessment in clinical practice. Treatment outcomes could improve with better identification of pain behaviors by veterinarians, technicians and even cat owners.

With the exception of day 1, food was withdrawn two hours after feeding which could underestimate actual daily food intake. The 2-hour interval was chosen to minimize bias over the next food intake evaluation especially for those cats that prefer fresh meals. Soft and dry food intake during 3 minutes, and dry food intake during two hours were significantly decreased in the severe disease group compared with those with minimal disease. This finding indicates that cats with severe disease may take longer to eat before and after dental extractions than cats with less severe disease. Additionally, this study showed that dry food intake might induce pain in cats with severe oral disease since these changes were observed before dental extractions. This would further compromise feline welfare and quality of life of these patients. Nutritional assessment via time taken for soft and dry food intake could be a useful indicator of oral pain and used in clinical studies. This study suggests that soft food should be offered to cats after multiple dental extractions for at least one week after surgery.

The concentrations of serum inflammatory cytokines in cats with oral disease were evaluated herein. However, other concomitant inflammatory conditions could have influenced our results. For example, degenerative joint disease (DJD) is a common disease in cats that causes inflammation and increases the concentrations of inflammatory biomarkers (238). In this clinical trial, the authors did not specifically investigate the presence or absence of DJD as part of our inclusion/exclusion criterion. Therefore, it is possible that some cats enrolled into the study had DJD and results could have been biased (238). Additionally, cats received a nonsteroidal anti-inflammatory drug until day 4 as part of multimodal analgesia. It is not known how meloxicam may have affected the concentrations of inflammatory cytokines. Nonetheless, baseline values should not have been affected since treatments were giving intra- and postoperatively. The pharmacokinetics of meloxicam have been described in cats (241), and the concentration of these biomarkers should not have been influenced by day 6 (two days after the last dose of the drug). Finally, both groups received the same dosage regimens and for the purpose of this study, results are comparable. In humans, concentrations of IL-1, IL-6 and TNF-α in the gingival crevicular fluid are known to contribute to acute and chronic inflammation in periodontal disease (220-222). In the current study, IL-6 was significantly different between days 0 and 6 in severe group. Overall, the concentrations

of some inflammatory biomarkers were lower at day 6 when compared with baseline values in the severe group showing that inflammation somehow subsided days after dental extractions. However, postoperative pain scores remained high in this group when compared with those with minimal disease at day 6 showing that the association between pain scoring and inflammatory biomarkers is not clear. Meanwhile, other cytokines (i.e. SCF) were also able to differentiate the two groups at baseline, while some (i.e. sFAS, IL-6, SDF-1, MCP-1) were significantly associated with dental parameters (i.e. number of teeth resorption, missing teeth and teeth fracture). These biomarkers could be possibly associated with the pathogenesis of oral disease and further investigation is warranted. A current study in our laboratory is investigating the relationship between local (i.e. tissue biopsy) and serum inflammatory biomarkers in cats with severe periodontal disease.

One potential limitation of this study was the lack of randomization and the use of a scoring system to determine group disease severity. The allocation of cats was ultimately based on the number of dental extractions. However, the scoring system was able to differentiate the two groups based on severity particularly when prevalence of rescue analgesia is considered. Additionally, age, body weight, anesthesia and surgery times were significantly different between groups. Indeed, age and body weight were previously correlated with the severity of periodontal disease (130). These factors (i.e. age, body weight, anesthesia and surgery) might have biased pain scoring by the observer and limited the findings of this study since one could infer disease severity based on surgery and anesthesia times. On the other hand, the authors improved the robustness of the study design by including objective outcome measures such as food intake and the evaluation of serum inflammatory cytokines.

Conclusion

This study showed that severe oral disease and multiple dental extractions produce severe pain and inflammation that require long-term analgesic treatment. Opioids were often required for up to 2 days after surgery. This condition affects food intake with an ultimate consequence for the welfare and nutritional status of these patients. Pain scores and inflammatory biomarkers were associated with dental parameters and could predict

postoperative analgesic requirements. The concentrations of serum inflammatory biomarkers after dental extractions and between severity groups were described and could provide future insights into the pathogenesis of oral disease in cats.

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Supporting Information

S1 File. Raw data (https://doi.org/10.1371/journal.pone.0213195.s001)

Article 2: Pain behaviors before and after treatment of oral disease in cats using video assessment: a prospective, blinded, randomized clinical trial

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Authors' contributions

RW: Collection and analysis of data, draft of the first version of the manuscript.

DF: Video analysis.

PS: Conceptualization, funding acquisition, development of methodology, manuscript preparation, the principal investigator of the study.

Abstract

Specific behaviors associated with pain in cats with oral disease have not been consistently studied. The aim of this exploratory study was to identify pain-induced behaviors in cats before and after treatment of oral disease using video assessment. Twenty-four cats (6 ± 3.3 years old; 4.9 ± 1.7 kg) were included in a prospective, blinded, randomized clinical trial. Cats were equally divided into minimal (G1: minimal dental treatment) or severe (G2: multiple dental extractions) oral disease groups. After acclimation at day 0, they underwent oral examination, radiographs, scaling, and dental extractions under general anesthesia (anesthetic protocol: acepromazine, hydromorphone, propofol, isoflurane, meloxicam, and local anesthetic blocks; day 1), and were discharged at day 6. Cats were filmed remotely for 10 min using a wide-angle glass lens camera before surgery (baseline) and throughout the study at different time points (36 h of video recording). The videos consisted of four parts namely general, playing, feeding and post-feeding behaviors. A board-certified behaviorist evaluated the duration/ frequency of different behaviors based on an ethogram, which were analyzed using linear mixed models and a generalized linear model, respectively ($p < 0.05$). In comparison with baseline, duration of “not pawing the face” was significantly shorter at day 3 in G2. These cats spent significantly longer time “standing” and “laying” at days 3 and 6, respectively; G1 spent significantly less time “walking” and “standing” at days 3 and 4, respectively and significantly longer time “immobile” at day 3. Duration of “no/slow tail movement” was significantly longer in G2 than G1 at day 5. Duration of “pawing the ribbon” (playing) was significantly shorter in G2 than G1 at day 1. Feeding and post-feeding behaviors with soft food were not significantly different between groups or over time. Frequency of “difficulty grasping dry food” was significantly higher in G2 than G1 up to day 6. Frequency of post-feeding “head shaking” was significantly higher in both groups at day 6 when compared with baseline. This study identified pain-induced behaviors in cats undergoing treatment of oral disease. These behaviors may be used to differentiate painful versus pain-free cats in clinical practice.

Introduction

Pain and periodontal disease affect quality of life in both humans and animals (41,178). Periodontal disease is one of the most commonly reported diseases in companion animals (48,52). In cats, it produces pain, inflammation, dysphagia, halitosis, weight loss and oral hemorrhage; full-mouth extractions are commonly required as treatment (218). However, behavioral signs of oral disease-induced pain have not been systematically investigated in cats. Current knowledge is mostly based on anecdotal evidence and review articles by experts (60,130,242), or studies performed in other species (128,243,244). If signs of pain are not recognized, dental disease and associated pain may result in treatment delay (i.e. dental cleaning, extractions, etc.) until pain is severe, and when there is a substantial impact on the cat's nutritional/welfare status (article 1). Additionally, it is not known how behaviors associated with oral pain differ between painful and non-painful cats, and how they are affected by treatment of oral disease.

The objectives of this study were to identify the specific pain-induced behaviors associated with oral disease in cats and to evaluate the effect of oral treatment (i.e. dental extractions) on these behaviors. The hypotheses were that cats with severe disease would present specific pain behaviors that would differ in duration and frequency from cats with minimal oral disease. In addition, dental extractions would produce postoperative pain and induce the appearance of new behaviors. This study was an exploratory study, and this manuscript represents a follow-up report on a recent publication where pain scores and prevalence of rescue analgesia, food intake, changes in inflammatory biomarkers, and the correlation between pain and the number of tooth extractions, gingival and calculus index were studied before and after oral treatment in cats with minimal and severe disease (article 1).

Materials and methods

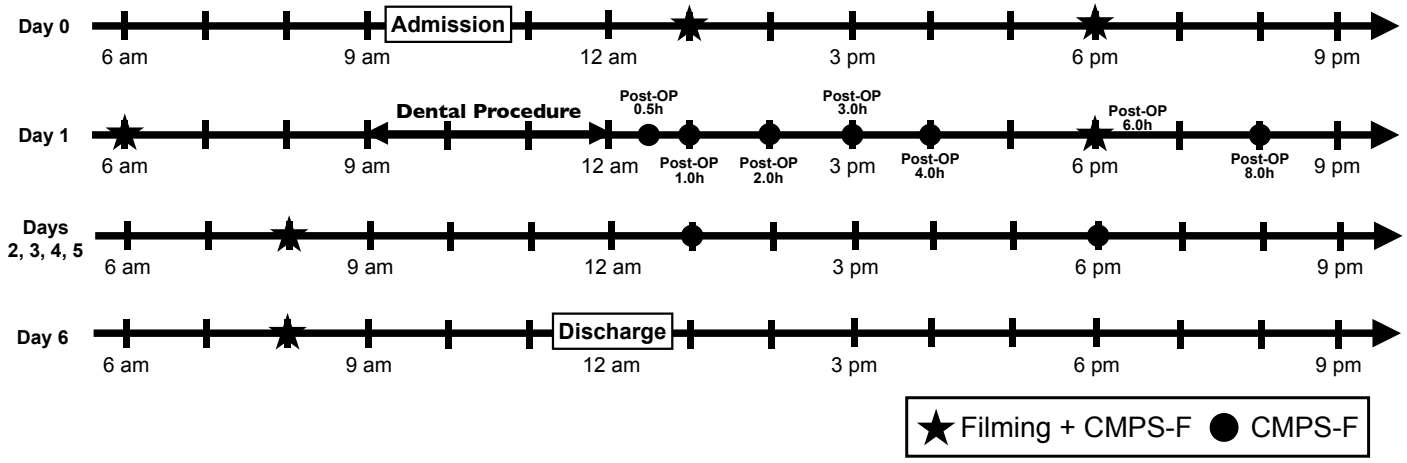
Study design

This study was approved by the Institutional Animal Care and Use Committee of the Université de Montréal (protocol 17-Rech-1890) and performed at the Centre hospitalier universitaire vétérinaire (CHUV), Faculty of Veterinary Medicine, Université de Montréal, between July 2017 and February 2018. This clinical trial is reported in accordance with the CONSORT guidelines (227). The study design was a prospective, blinded, randomized clinical trial.

Animals

Twenty-four adult (> 1 year of age) cats of any breeds and gender with or without naturally occurring oral disease were included. Cats that were considered as a need to evaluate the oral conditions were recruited from different shelter facilities. Before enrollment, their oral conditions including the condition of gingiva and the amount of calculus were evaluated by the dentistry service so the principal investigator (PS), but not other observers involved with anesthesia and pain assessment, would have an idea of group allocation that could facilitate further patient recruitment (cats with minimal or severe disease). A written informed consent was obtained before enrolment in the study. Animals were admitted approximately 24 hours before general anesthesia (day 0); dental treatment was performed on day 1. Cats were discharged on day 6 (7 days after arrival and 6 days after treatment of oral disease) (Figure 1). During hospitalization, they were housed in stainless steel cages in the cat ward of the CHUV with access to water ad libitum, toys, litter box and bedding.

Figure 1. Example of a timeline for pain assessment and video filming in cats undergoing oral treatment for 7 days. CMPS-F: Glasgow Composite Measure Pain Scale-Feline



Inclusion and exclusion criteria

Cats with body condition score ranging from 4 to 6 out of 9 and with no/minimal or severe oral disease that would require oral treatment including dental examination, scaling and/or extractions were included in the study. Inclusion criteria were also based on history, medical records, complete physical examination, and hematology and biochemical panel. Cats presenting fearful behaviors, concurrent medical conditions, systemic disorders (e.g. cancer, renal, cardiovascular, hepatic, or gastrointestinal disease) were not included. Cats were excluded if they received any medication including analgesics and antibiotics for up to 10 days before the study had begun or presented signs of disease during hospitalization.

Treatment of oral disease

Group allocation

Complete dental examination and radiography were performed, and patients underwent dental scaling and dental extractions (if needed) by a board-certified dentist and a resident of the American Veterinary Dental College. Group allocation (i.e. minimal or severe oral disease) was determined according to a scoring system suggested by these two

individuals in agreement with the principal investigator (PS) based on their previous clinical experience. In brief, the number and location of extractions were thought to be important in determining the possible severity of postoperative pain (article 1). The scores were as follows: canine tooth - 3 points, third premolar of maxilla or molar of mandible - 2 points, second premolar of maxilla or premolar of mandible - 1 point; a score of 2 points was given if seven or more incisive teeth and/or first premolars of the mandible were extracted; otherwise a score of 1 point was given if six or fewer incisive teeth were removed. The total dental score was calculated, and cats were allocated to the minimal oral disease group if dental score ≤ 7, and to the severe oral disease group if dental score was ≥ 8.

Anesthesia and analgesic protocol

Premedication consisted of intramuscular (IM) (i.e. epaxial muscles) administration of acepromazine (0.02 mg/kg; 1 mg/mL, Acepromazine maleate, Gentès & Bolduc, Saint-Hyacinthe, QC, Canada) and hydromorphone (0.1 mg/kg; 2 mg/mL, Hydromorphone hydrochloride, Sandoz, Boucherville, QC, Canada). A eutectic mixture of local anesthetic cream (EMLA cream lidocaine 2.5% and procaine 2.5% cream, Astra Zeneca, Mississauga, ON, Canada) was applied to the skin over the cephalic vein after clipping and covered with plastic film and adhesive bandage. Approximately 20 minutes later, a 22-G intravenous (IV) catheter was aseptically placed in one of the cephalic veins. Anesthetic induction was performed with intravenous propofol (10 mg/mL, Propoflo 28, Zoetis, Kirkland, QC, Canada) until the anesthetic depth for endotracheal intubation was achieved (4.0 ± 1.2 mg/kg). The arytenoid cartilages were splashed with 0.05 mL of lidocaine 2% (Lidocaine hydrochloride sterile injection, 20 mg/mL, Vétoquinol N.-A.Inc, Lavaltrie, QC, Canada), and cats were intubated with a cuffed endotracheal tube and connected to a coaxial Mapleson D system. Anesthetic maintenance was performed with isoflurane (Isoflurane USP, Fresenius Kabi, Toronto, ON, Canada) in 100% oxygen. Anesthetic monitoring was performed with a multiparametric monitor (Lifewindow 6000V Veterinary Multiparameter Monitor; Digicare Animal Health, Boynton Beach, FL, USA) as reported in our previous article (article 1). A crystalloid solution was administered (2-5 mL/

kg/hour) throughout the procedure. Cats received local anesthetic blocks with bupivacaine 0.5% (5 mg/mL, Sensorcaine, AstraZeneca, ON, Canada) using a 25-G needle if dental extractions were required. These included the mental, infraorbital, maxillary and/or inferior alveolar mandibular nerve blocks approximately 20 minutes before tooth extraction. The total dose of bupivacaine for all anesthetic blocks did not exceed 2 mg/kg. Meloxicam (0.2 mg/kg; Metacam 5 mg/mL Solution for Injection; Boehringer Ingelheim, Burlington, ON, Canada) was administered subcutaneously at the end of the dental procedure. Three additional doses of meloxicam at 0.05 mg/kg were administered orally at 24, 48 and 72 hours after the first dose according to label recommendations in Canada.

Video recording

The schedule for video recording was performed according to Figure 1. There were nine time points of video recording and each lasted 10 minutes (total of 90 minutes for each cat). A wide-angle glass lens camera (GoPro Hero 5, GoPro, Riverside, CA, USA) set between cage bars was used. Cats were moved to a specific cage for video recording that included better lighting and material quality. After a 5-minute acclimation period, the camera was activated remotely using a smart-phone (iPhone7, Apple Inc, Cupertino, CA, USA). During the 10-minute period, video recording was performed as follows: a) time 0-3 minutes: the general behaviors of the cat were recorded without any observer in the room (3 minutes; general behavior), b) time 3-5 minutes: the observer entered the room, greeted and petted the cat, stimulated the cat to play with a ribbon toy (2 minutes; playing behavior), c) time 5-8 minutes: the cat was fed with dry or soft food; feeding should potentially evoke pain behaviors as it has been described in the literature (3 minutes; feeding behavior) (130) and d) time 8-10 minutes: food was removed, and cats were filmed for another 2 minutes without the observer in the room (2 minutes; post-feeding behavior). Cats were fed with dry food (Hill's Science Diet, Adult Optimal Care – Dry; Hill's Pet Nutrition Canada Inc., Mississauga, ON, Canada) at 6 pm on day 0 and 8 am on day 6. A commercial canned prescription recovery diet (Hill's Prescription Diet a/d; Hill's Pet Nutrition Canada Inc., Mississauga, ON, Canada) was provided at 1 pm on day 0; 6 am

and postoperative 2 and 6 hours on day 1; at 8 am, 1 pm and 6 pm at days 2, 3, 4 and 5. Any remaining food was removed after 2 hours.

Video analysis

A total of 36 hours of video material was analyzed using a professional software (The Observer XT, Noldus information technology, VA, U.S.A). Videos were randomized according to the website www.randomization.com and assessed by a board-certified behaviorist [DF] of the American College of Veterinary Behaviorists who was blinded to severity groups. An ethogram was developed using normal behaviors and those described in painful cats with oral disease in review and scientific articles, textbooks and clinical experience (8,60,130). Some behaviors were added to the ethogram based on the researchers' observation during pain assessment of these cats (article 1). The duration (%) (duration of each behavior/video length × 100) or frequency (times of the event/minute or total number of each behavior during the video/video length) for each behavior were obtained for statistical analysis. Baseline duration and frequency of each behavior were calculated using the mean of preoperative values. For general and playing behaviors, the mean of three values were used (1 pm and 6 pm on day 0 and 6 am on day 1) whereas for feeding and post-feeding with soft food, the mean of two values (1 pm on day 0 and 6 am on day 1) were used to calculate baseline mean values. The behaviors that were recorded less than five times during video assessment were excluded from statistical analysis.

Pain assessment

Pain assessment was performed by an observer [RW] who was unaware of the disease severity using the Glasgow composite measure pain scale-feline (CMPS-F) according to Figure 1 (121). Pain assessment was performed before video recording. Rescue analgesia was administered with hydromorphone at 0.05 mg/kg IV (if the intravenous catheter was in place, first 24 hours after surgery) or 0.1 mg/kg IM (if the intravenous catheter had been removed) when CMPS-F scores were ≥ 5/20 at any time during the

study. Based on the duration of hydromorphone in cats, the videos obtained within 2 hours of rescue analgesia were excluded from statistical analysis to avoid bias (245).

Statistical analysis

Statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC, USA). The power analysis revealed that eight cats would be needed per group to detect a difference of three points in the CMPS-F pain scores between the two groups 80% of the time using an alpha value of 0.05, and a standard deviation within group of two points (article 1). Twelve cats were included per group for adequate power considering the individual variability of oral disease. After normality test using a Shapiro-Wilk test, demographic data for each treatment group were compared using two-sample t-tests or Mann-Whitney U where appropriate. Duration and frequency of each behavior were compared between groups at each time point, and between baseline and the postoperative time points in both groups. Duration of each behavior was transformed using the arcsine square root transformation and analyzed using a linear mixed model with patient identification as the random factor, and groups and time and their interaction as fixed factors, and gender as co-factor. Frequency of each behavior was analyzed using a generalized linear model with log link and Poisson errors with patient identification as the random factor, groups and time as fixed factors, and gender as co-factor. When there was an association with fixed factors, a series of a priori contrasts were performed to compare the means using sequential Benjamini-Hochberg's adjustment. $p < 0.05$ was considered statistically significant.

Results

Descriptive statistics for age, body weight, body condition score, dental score and number of extracted teeth are presented in Table 1. Cats in the minimal oral disease group were younger and lighter than those in the severe oral disease group as previously reported (Table 1) (article 1).

Table 1. Demographic data, dental score and number of extracted teeth in cats with minimal or severe oral disease.

Variable	Minimal (n = 12)	Severe (n = 12)	p value
Gender (male, female)	Male: 3, Female: 9	Male: 9, Female: 3	
Breed	Domestic short-hair: 11 Siamese: 1	Domestic short-hair: 9 Domestic long-hair: 3	
Age (years)	3.6 (2.0)	8.5 (2.2)	< 0.0001
Body weight (kg)	4.0 (0.6)	5.8 (1.9)	0.007
Body condition score (1-9)	5 (5-6)	6 (4-6)	0.078
Dental score	1 (0-4)	17 (8-28)	< 0.0001
Number of extracted teeth	2 (0-5)	17 (8-30)	< 0.0001

Values are expressed as mean (SD) with exception of body condition score, dental score and number of extracted teeth which are reported as median (min-max)

One cat from the minimal disease group was excluded in the postoperative period due to wound dehiscence, and only preoperative data of this individual were included in the analysis. A total of 11 out of 12 cats (91.7%) in severe group received rescue analgesia on the day of dental procedure (day 1). Five videos obtained at postoperative 6 hours were excluded from the statistical analysis.

The ethogram and the behaviors with low frequency (fewer than five times over a minute of observation) during video analysis that were excluded from statistical analysis are shown in Tables 2 and 3, respectively.

Appendix 1 presents the p values for duration and frequency of some behaviors that were not statistically associated with fixed factors (i.e. group, time, group X time and gender).

Tables 4, 5 and 6 show the duration (%) of general and playing behaviors and frequency (times/minute) of behaviors in cats with minimal or severe oral disease before and after treatment, respectively.

Table 2. Ethogram of general, playing, feeding and post-feeding behaviors

General		Playing	Feeding	Post-feeding
Position in the cage (D)	Back	Pawing (D)	Eating food (D)	Grooming (D)
	Front	No pawing (no interest) (D)	Not eating food (D)	Lip licking (D)
Attention to the surroundings (D)	Looking around front of the cage	No pawing but attention to ribbon (D)	Tongue flicking (D)	Mouth pawing (D)
	Not looking around front of the cage	No pawing but attention to observer (D)	Vocalization (meowing) (F)	No grooming, mouth pawing, lip licking (D)
Activity (F)	Pawing the face	No pawing with looking away from ribbon (D)	Growling (F)	Tongue flicking (D)
	Not pawing the face	Chewing ribbon (D)	Jaw quivering (F)	Teeth chattering (F)
	Lip licking	Grabbing ribbon in mouth (F)	Ptyalism (F)	Jaw quivering (F)
	Yawning		Difficulty grasping food (F)	Mouth opening (F)
	Swallowing		Dropping food (F)	Head shaking (F)
	Vocalization		Head shaking (F)	Yawning (F)
	Tongue flicking		Tongue flicking (F)	Vocalization (F)
Movement (D)	Walking		Mouth opening (F)	Swallowing (F)
	Immobile		Yawning (F)	Tongue flicking (cat did not eat) (F)
Body position (D)	Sitting		Lip licking not related to eating (F)	
	Standing		Swallowing not related to eating (F)	
	Laying		Vocalization not related to eating (F)	
	Crouching			
Tail position (D)	Up			
	Curling around feet/body			
Tail movement (D)	Swishing			
	Not or slow movement			
Activity (D)	Stretching			
	Grooming			
	Not stretching and grooming			

(D) and (F) indicate the duration and frequency, respectively.

Table 3. The behaviors with low frequency (fewer than five times over a minute of observation) during video analysis that were excluded from statistical analysis

General behaviors	Feeding (soft food)	Feeding (dry food)	Post-feeding (soft food)	Post-feeding (dry food)
Pawing the face	Tongue flicking	Tongue flicking	Mouth pawing	Mouth pawing
Tail up	Vocalization (meowing)	Vocalization (meowing)	Tongue flicking	Tongue flicking
Tail swishing	Growling	Growling	Teeth chattering	Teeth chattering
Stretching	Jaw quivering	Jaw quivering	Jaw quivering	Jaw quivering
	Ptyalism	Ptyalism	Mouth opening	Mouth opening
	Difficulty grasping food	Head shaking	Yawning	Yawning
	Dropping food	Tongue flicking	Swallowing	Swallowing
	Head shaking	Mouth opening	Tongue flicking	Tongue flicking
	Mouth opening	Yawning		
	Yawning	Swallowing not related to eating		
	Swallowing not related to eating	Vocalization not related to eating		
	Vocalization not related to eating			

Table 4. Mean (SD) of duration (%) of general behaviors in cats before and after dental treatment

Action category	Individual behavior	Time point	Minimal	Severe	p value between groups	p value compared with baseline	
						Minimal	Severe
Position in the cage	Back	Baseline	8.9 (12.1)	30.3 (36.5)	0.028*		
		Day 1	10.9 (30.2)	10.4 (19.9)	0.913	0.717	0.069
		Day 2	3.9 (13.0)	24.6 (40.6)	0.014*	0.211	0.426
		Day 3	0.0 (0.0)	16.7 (38.9)	0.031*	0.084	0.080
		Day 4	6.1 (20.2)	10.1 (20.9)	0.200	0.276	0.020*
		Day 5	1.0 (2.8)	16.7 (38.9)	0.049*	0.155	0.090
		Day 6	0.4 (1.4)	10.7 (17.3)	0.086	0.113	0.026*
	Front	Baseline	91.3 (12.1)	69.7 (36.5)	0.028*		
		Day 1	89.1 (30.2)	89.6 (19.9)	0.913	0.717	0.007*
		Day 2	96.1 (13.0)	75.5 (40.6)	0.014*	0.211	0.426
		Day 3	100.0 (0.0)	83.3 (38.9)	0.031*	0.084	0.080
		Day 4	93.9 (20.2)	89.9 (20.9)	0.200	0.276	0.020*
		Day 5	99.0 (2.8)	83.3 (38.9)	0.049*	0.155	0.090
		Day 6	99.6 (1.4)	89.3 (17.3)	0.086	0.113	0.026*
Attention to surroundings	Looking around front of the cage	Baseline	99.6 (1.3)	77.6 (29.4)	0.003*		
		Day 1	96.6 (11.4)	74.7 (40.0)	0.023*	0.800	0.905
		Day 2	86.5 (27.2)	69.6 (37.3)	0.021*	0.157	0.444
		Day 3	99.6 (1.2)	82.1 (27.8)	0.013*	0.948	0.602
		Day 4	94.9 (17.1)	90.6 (20.2)	0.188	0.700	0.131
		Day 5	98.9 (3.6)	83.8 (28.5)	0.034*	0.969	0.374
		Day 6	100.0 (0.0)	90.9 (28.7)	0.105	0.836	0.081
	Not looking around front of the cage	Baseline	0.4 (1.3)	22.5 (29.4)	0.003*		
		Day 1	3.4 (11.4)	25.3 (40.0)	0.023*	0.800	0.905
		Day 2	13.5 (27.2)	30.4 (37.3)	0.021*	0.157	0.444
		Day 3	0.4 (1.2)	17.9 (27.8)	0.013*	0.948	0.602
		Day 4	5.2 (17.1)	9.4 (20.2)	0.188	0.701	0.131
		Day 5	1.1 (3.6)	16.2 (28.5)	0.034*	0.969	0.374
		Day 6	0.0 (0.0)	9.1 (28.7)	0.105	0.836	0.081
Activity	Not pawing the face	Baseline	88.3 (9.7)	85.3 (14.4)	0.328		
		Day 1	85.5 (25.2)	87.5 (15.3)	0.839	0.553	0.137
		Day 2	89.6 (19.6)	74.5 (35.4)	0.017*	0.227	0.292
		Day 3	85.7 (28.5)	98.3 (3.0)	0.620	0.440	**0.003**
		Day 4	86.6 (17.3)	95.1 (5.5)	0.957	0.579	0.049*
		Day 5	82.2 (21.4)	89.8 (17.2)	0.853	0.646	0.189
		Day 6	87.6 (25.8)	85.4 (24.8)	0.342	0.382	0.367
Movement	Walking	Baseline	2.1 (1.4)	1.7 (1.8)	0.918		
		Day 1	1.3 (1.9)	4.5 (8.7)	0.266	0.056	0.846
		Day 2	1.4 (2.2)	1.4 (3.0)	0.728	0.065	0.187
		Day 3	0.4 (1.0)	0.0 (0.0)	0.889	**0.002**	0.004*
		Day 4	0.9 (2.5)	1.0 (1.6)	0.298	0.007*	0.183
		Day 5	1.5 (2.6)	0.7 (1.5)	0.863	0.101	0.075
		Day 6	1.8 (2.9)	2.7 (5.2)	0.371	0.105	0.716
	Immobile	Baseline	97.9 (1.4)	98.3 (1.8)	0.919		
		Day 1	98.7 (1.9)	95.5 (8.7)	0.266	0.056	0.846
		Day 2	98.6 (2.2)	98.6 (3.0)	0.728	0.065	0.187
		Day 3	99.6 (1.0)	100.0 (0.0)	0.888	**0.002**	0.004*
		Day 4	99.1 (2.5)	99.0 (1.6)	0.298	0.007*	0.183
		Day 5	98.5 (2.6)	99.3 (1.5)	0.863	0.101	0.075
		Day 6	98.2 (2.9)	97.3 (5.2)	0.371	0.105	0.716
Body position	Sitting	Baseline	85.0 (14.1)	58.0 (40.2)	0.037*		

		Day 1	63.3 (37.5)	70.8 (40.6)	0.914	0.194	0.158
		Day 2	71.3 (43.6)	58.6 (44.6)	0.166	0.516	0.811
		Day 3	83.6 (31.0)	83.3 (38.9)	0.499	0.552	0.013*
		Day 4	83.7 (31.5)	89.6 (20.6)	0.799	0.661	0.004*
		Day 5	80.0 (26.5)	76.8 (39.3)	0.396	0.091	0.075
		Day 6	87.5 (23.3)	71.8 (40.1)	0.149	0.515	0.136
		Baseline	5.6 (3.8)	3.9 (4.7)	0.640		
		Day 1	2.5 (4.5)	2.2 (3.6)	0.860	0.009*	0.115
		Day 2	2.1 (3.4)	3.4 (7.0)	0.480	0.008*	0.210
	Standing	Day 3	2.6 (6.2)	0.0 (0.0)	0.521	0.006*	**0.002**
		Day 4	1.4 (3.1)	1.8 (2.9)	0.357	**0.001**	0.121
		Day 5	3.7 (5.4)	2.0 (5.2)	0.792	0.054	0.081
		Day 6	3.2 (5.1)	5.8 (9.8)	0.256	0.026*	0.825
		Baseline	0.1 (0.2)	29.0 (38.2)	0.015*		
		Day 1	15.3 (34.8)	24.6 (41.5)	0.798	0.105	0.235
		Day 2	9.1 (30.2)	30.2 (45.5)	0.063	0.329	0.805
	Laying	Day 3	0.0 (0.0)	16.7 (38.9)	0.108	0.939	0.224
		Day 4	0.0 (0.0)	7.4 (18.8)	0.379	0.939	0.023*
		Day 5	0.0 (0.0)	8.3 (28.9)	0.352	0.939	0.028*
		Day 6	0.0 (0.0)	0.2 (0.8)	0.745	0.939	**0.002**
		Baseline	33.2 (31.2)	14.5 (23.1)	0.719		
		Day 1	37.4 (46.3)	21.2 (40.1)	0.821	0.859	0.804
		Day 2	7.6 (17.7)	0.0 (0.0)	0.653	0.035*	0.212
Tail position	Curling around feet/body	Day 3	18.2 (40.1)	25.0 (45.2)	0.140	0.190	0.428
		Day 4	18.8 (40.2)	16.1 (33.0)	0.436	0.228	0.936
		Day 5	8.1 (26.8)	20.4 (39.3)	0.052	0.029*	0.666
		Day 6	9.4 (29.1)	8.3 (28.9)	0.355	0.046*	0.569
		Baseline	54.4 (20.6)	45.0 (30.6)	0.902		
		Day 1	73.2 (40.4)	62.4 (43.5)	0.489	0.217	0.882
		Day 2	47.2 (50.5)	40.0 (48.5)	0.856	0.680	0.723
Tail movement	No or slow movement	Day 3	58.0 (47.1)	50.0 (52.2)	0.816	0.862	0.750
		Day 4	45.4 (52.1)	51.5 (50.9)	0.334	0.573	0.643
		Day 5	22.9 (35.7)	77.2 (41.7)	**0.001**	0.078	0.039*
		Day 6	45.3 (44.6)	49.0 (46.7)	0.515	0.655	0.844

* not significant after adjustment

Table 5. Mean (SD) of duration (%) of playing behaviors in cats before and after dental treatment

Individual behavior	Time point	Minimal	Severe	p value between groups	p value compared with baseline	
					Minimal	Severe
Pawing ribbon	Baseline	45.0 (31.8)	15.7 (19.8)	0.018*		
	Day 1	46.7 (37.2)	0.4 (0.7)	**< 0.001**	0.943	0.004*
	Day 2	27.1 (25.7)	11.9 (17.5)	0.143	0.003*	0.300
	Day 3	39.4 (36.9)	14.9 (20.3)	0.054	0.208	0.739
	Day 4	33.4 (35.1)	11.4 (15.1)	0.086	0.034	0.462
	Day 5	36.7 (36.6)	12.4 (18.1)	0.059	0.087	0.474
	Day 6	33.4 (29.5)	5.9 (15.2)	0.013*	0.064	0.025*
No pawing but attention to ribbon	Baseline	13.6 (15.9)	22.3 (12.5)	0.031*		
	Day 1	10.0 (15.9)	15.3 (20.1)	0.0001*	0.610	0.002*
	Day 2	10.0 (18.1)	14.6 (16.9)	0.016*	0.718	0.748
	Day 3	5.6 (10.2)	15.4 (15.8)	0.016*	0.672	0.808
	Day 4	3.9 (5.9)	22.4 (24.3)	0.026*	0.506	0.665
	Day 5	4.8 (8.1)	16.9 (19.5)	0.024*	0.405	0.580
	Day 6	2.7 (4.1)	19.9 (23.8)	0.010*	0.427	0.824

* not significant after adjustment

Table 6. Mean (SD) of frequency (times/min) of behaviors in cats before and after dental treatment

	Individual behavior	Time point	Minimal	Severe	p value between groups	p value compared with baseline	
						Minimal	Severe
Feeding (dry)	Difficulty of grasping food	Baseline	0.3 (0.6)	1.3 (1.8)	**0.005**		
		Day 6	0.2 (0.7)	2.0 (2.1)	**0.001**	0.376	0.156
Post-feeding (dry)	Head shaking	Baseline	0.1 (0.3)	0.2 (0.4)	0.622		
		Day 6	0.5 (0.7)	0.7 (1.1)	0.733	**0.001**	**0.005**

General behavior

In comparison with baseline, duration of “not pawing the face” was shorter at day 3, and “standing” and “laying” were longer at days 3 and 6, respectively in the severe group; duration of “walking” was shorter at day 3, “immobile” was longer at day 3 and “standing” was shorter at day 4 in the minimal group (Table 4). Duration of “no/slow tail movement” was longer in the severe than in the minimal group at day 5 (Table 4). The expected occurrence of duration of “tail curl” was significantly higher in female than male (p = 0.017).

Playing behavior

Duration of “pawing the ribbon” was significantly shorter in the severe group than in the minimal group at day 1 (Table 5).

Feeding behavior

Dry food

Cats in the severe group had significantly higher frequency of “difficulty grasping dry food” than in the minimal group up to day 6 (Table 6). This specific behavior was observed more commonly in males than females ($p = 0.029$).

Post-feeding behavior

Dry food

Frequency of post-feeding “head shaking” was significantly higher in both groups at day 6 when compared with baseline (Table 6).

A supplementary material (Appendix 2) includes a video with a summary of behavior changes and results of the study in cats with minimal or severe oral disease.

Discussion

This study identified specific pain-induced behaviors associated with oral disease in cats undergoing dental treatment. According to our hypotheses, these behaviors differed between cats with minimal and severe oral disease, and new behaviors appeared after the dental procedure due to postoperative pain (article 1). Overall, cats with severe oral disease were less active when compared with baseline or cats with minimal oral disease. For example, duration of “walking” and “standing” was shorter whereas they were more reluctant to move (“immobile” and “no/slow tail movement”) than in the minimal group at specific time points postoperatively. Additionally, postoperative pain induced changes in

grooming. Duration of “not pawing the face” was shorter in cats with severe oral disease after the dental procedure than baseline. Less activity was also observed with these cats: duration of “standing” and “laying” was longer after dental extractions than before the procedure.

Some studies have evaluated oral pain when comparing the efficacy of different analgesic treatments in dogs and cats (8,246). In the current study, the CMPS-F was used for pain assessment. This tool has been widely used for feline acute pain evaluation, and theoretically, it can be applied for different sources of pain (121). The UNESP-Botucatu multidimensional composite pain scale for feline pain assessment has only been validated in cats undergoing ovariohysterectomy and the authors opted to use the CMPS-F in this study (119). However, the authors found some limitations when using the CMPS-F to evaluate oral pain in this study. None of the cats scored points for questions 3 (ignoring any wound or painful area: 0 points or attention to wound: 1 point) or 6 (after gentle pressure of the wound, does the cat?: do nothing – 0 points; swish tail/flatten ears – 1 point; cry/hiss – 2 points; growl – 3 points; bite/lash out – 4 points). Therefore, it may be difficult to predict how cats would give attention to wound for question 3 in dental pain. Indeed, an opposite finding would be expected when cats are painful. Additionally, most cats do not appreciate palpation of the mouth area before or after the dental procedure for question 6. An escape behavior was often noticed but none of the behaviors of CMPS-F question 6 was easily detected. Based on this rationale, it is possible that pain was underestimated in some cats when they were less active and reluctant to move. The pain-induced behaviors reported here may add additional information to feline pain assessment in dentistry and clinical practice.

The study presented an ethogram of normal and those behaviors that are presumed to be affected by oral disease based on previous reports and clinical experience (8,60,130). However, some of these behaviors are also known to be influenced by the cats’ demeanor (247). For this reason, cats with shy or fearful behavior were excluded to minimize bias and overestimation of pain scores during assessment.

The duration of “pawing the ribbon” was significantly shorter in the severe group than in the minimal group. Additionally, albeit not significantly, the duration of “no pawing but

attention to ribbon" was always longer in severe than in the minimal group. These playing behaviors were affected by oral pain after dental treatment; painful cats with severe oral disease were less playful. On the other hand, playing is a unique feature of each cats' demeanor and temperament, which could be affected by pain, but also stress, anxiety and hospitalization. This may be the reason why the duration of other playing behaviors was not always significantly different between groups or baseline values (i.e. "chewing the ribbon" and "grabbing in the mouth"). Therefore, changes in playing behavior may be more important in the home environment than in the hospital setting.

Cats with severe oral disease showed significant differences in feeding behavior when compared with cats with minimal disease. These differences were also observed in both groups for "head shaking" during post-feeding behavior assessment on day 6. The behavior "head shaking" was probably evoked by pain during feeding since severe acute inflammation is present in the first postoperative days. Chewing the dry food by using the remaining teeth but also the gingiva/wound where teeth were extracted may produce pain. Our previous study showed that the amount of dry and soft food intake for 3 minutes, and dry food intake for 2 hours were significantly decreased in cats with severe oral disease. (article 1). The study concluded that cats with oral pain require longer periods of time to eat both dry and soft food than those with minimal pain. Frequency of "difficulty grasping dry food" was observed more commonly in males than females. This could be explained by the unequal distribution of male and female cats in the study (3 males and 9 females in minimal group and 9 males and 3 females in severe group). Therefore, this result may show that cats with severe disease had more "difficulty grasping dry food" than those with minimal disease, and may not have a direct association with sex per se.

This study has some limitations: 1) palpation of the painful area (question 6 of CMPS-F) was performed over the lips since direct palpation of gingiva would not always possible due to some cat's temperament. Additionally, this would have unmasked the observer to the dental severity group; 2) many behaviors were not significantly different between groups for duration and frequency. In this case, the number of behaviors analyzed using the ethogram and the rigorous statistical approach with many group comparisons followed by sequential adjustment resulted in a decrease of the significant "real" p value. It seems

that this is not a specific issue to our study or in cats, and it could be also related to duration of filming. For example, previous studies could not find significant differences in the frequencies of specific behaviors in rats or bears with oral pain when duration of filming was short (7 and 15 minutes, respectively) or similar to our study (10 minutes) (128,244). On the other hand, frequencies of oral pain behaviors were found in ferrets when using longer filming periods (1 hour for each time point) than the present study (244). Therefore, duration and frequency of other specific behaviors could exist in cats with oral disease if duration of filming was longer than in this study. 3) 11 out of 12 cats in severe group received rescue analgesia at day 1 when postoperative acute pain and inflammation is severe. Five of these videos of painful cats were excluded from the analysis after the administration of hydromorphone since this could have biased video assessment (245). This high prevalence of rescue analgesia in the severe group on day 1 may have underestimated our video observations. In other words, some differences could have been detected between disease severity groups, and day 1 in comparison with baseline if these videos had not been excluded. 4) there were several behaviors in the study that were no longer significant after statistical adjustment due to the numbers of comparisons. This could have led to a type II error where a difference between disease severity groups existed, but this hypothesis was rejected after sequential adjustment. Perhaps, this may be the main reason why some of the behaviors were not statistically significant even when they could be of clinical relevance. This included “position in the cage” (i.e. duration in the “back of the cage”), “attention to surroundings” (i.e. duration of “not looking around front of the cage”), and “body position” (i.e. “laying”), and playing behaviors (i.e. “pawing ribbon” and “no pawing with attention to ribbon”).

Conclusion

This study identified some pain-induced behaviors in cats undergoing treatment of oral disease that can be used to differentiate painful versus pain-free cats, and as indicators of acute pain in these patients. Overall, cats with severe oral disease were less active, less playful and had more difficulty grasping dry food.

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Supporting Information

Appendix 1. p values of behaviors that were not significantly associated with fixed factors. Table with individual behaviors and p values for group, time, group x time and gender comparisons.

	Individual behavior	Group	Time	Group × time	Gender
General	Lip licking	0.164	0.352	0.256	0.745
	Yawning	0.295	0.695	*	0.767
	Swallowing	0.217	0.523	0.790	0.838
	Vocalization	0.277	0.065	*	0.988
	Tongue flicking	0.329	0.194	0.567	0.514
	Crouching	0.973	0.914	0.186	0.407
	Grooming	0.293	0.331	0.780	0.156
	Not stretching and grooming	0.296	0.351	0.794	0.149
Playing	No pawing (no interest)	0.187	0.081	0.667	0.183
	No pawing but attention to observer	0.580	0.071	0.451	0.901
	No pawing with looking away from	0.756	0.929	0.385	0.996
	Chewing ribbon	0.065	0.163	0.185	0.566
	Grabbing ribbon in mouth	0.060	0.092	*	0.857
Feeding (soft food)	Eating food	0.585	0.170	0.566	0.362
	Not eating food	0.602	0.222	0.438	0.571
	Tongue flicking	0.265	0.485	*	0.204
	Lip licking not related to eating	0.928	0.150	*	0.210
Feeding (dry food)	Eating food	0.072	0.430	0.731	0.831
	Not eating food	0.137	0.318	0.838	0.799
	Dropping food	0.736	0.313	*	**0.029**
	Lip licking not related to eating	0.314	0.181	*	0.364
Post-feeding (soft)	Grooming	0.567	0.317	0.105	0.987
	Lip licking	0.857	0.726	0.929	0.646
	No grooming, mouth pawing, lip licking	0.946	0.736	0.329	0.506
	Head shaking	0.745	0.933	0.070	0.411

	Vocalization	0.165	0.292	0.374	0.340
Post-feeding (dry)	Grooming	0.700	0.055	0.374	0.947
	Lip licking	0.802	0.612	0.985	0.341
	No grooming, mouth pawing, lip licking	0.978	0.622	0.410	0.243
	Vocalization	0.411	0.706	*	0.385

Appendix 2. Summary of behavior changes and results of the study in cats with minimal or severe oral disease (mp4). 10-minute video consisted of 4 parts including general, playing, feeding and post-feeding behaviors. Cats with severe oral disease were less active, less playful and had more difficulty grasping dry food. (https://bmcvetres.biomedcentral.com/articles/10.1186/s12917-020-02302-w

Article 3: Inter-rater reliability of the Feline Grimace Scale in cats undergoing dental extractions

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Authors' contributions

Designing and conduction of the study: RW, PS

Writing (original draft): RW, PS

Postoperative care, pain assessment, the video filming, the image selection, the statistical analyses: RW

General anesthesia and the image captures: GD

Image scoring: ME, HR, BM, PS

Abstract

This study aimed to evaluate the inter-rater reliability of the Feline Grimace Scale (FGS) in cats undergoing dental extractions and the effects of the caregiver's presence on the FGS scores. Twenty-four cats (6 ± 3.3 years old; 4.9 ± 1.7 kg) undergoing oral treatment were included in a prospective, blinded, randomized, clinical study. They underwent treatment under general anesthesia (acepromazine-hydromorphone-propofol-isoflurane-meloxicam-local anesthetic blocks) at day 1 and were discharged at day 6. Images of cat faces were captured from video recordings with or without the caregiver's presence at 6 h postoperatively (day 1), day 6, and before and after rescue analgesia. Images were randomized and independently evaluated by four raters using the FGS [five action units (AU): ear position, orbital tightening, muzzle tension, whiskers change, and head position; score 0–2 for each]. Inter-rater reliability and the effects of the caregiver's presence were analyzed with intraclass correlation coefficient [single measures (95% confidence interval)] and the Wilcoxon signed-rank test, respectively ($p < 0.05$). A total of 91 images were scored. Total FGS scores showed good inter-rater reliability [0.84 (0.77–0.89)]. Reliability for each AU was: ears [0.68 (0.55–0.78)], orbital tightening [0.76 (0.65–0.84)], muzzle [0.56 (0.43–0.69)], whiskers [0.64 (0.50–0.76)], and head position [0.74 (0.63–0.82)]. The FGS scores were not different with [0.075 (0–0.325)] or without [0.088 (0–0.525)] the caregivers' presence ($p = 0.12$). The FGS is a reliable tool for pain assessment in cats undergoing dental extractions. The caregiver's presence did not affect FGS scores.

Introduction

Oral disease is often observed in veterinary medicine (177). Our laboratory revealed that cats with severe oral disease requiring multiple tooth extractions had specific pain-induced behaviors, higher pain scores, changes in serum inflammatory cytokines, and lower food intake when compared with cats with no/minimal oral disease (articles 1,2). There are three pain scales with validation for feline pain assessment: Glasgow Composite Measure Pain Scale-Feline (CMPS-F) (121), UNESP-Botucatu multidimensional composite pain scale (119) and the recent Feline Grimace Scale (123,248). However, these tools have not been used specifically in the context of pain caused by oral disease. The main challenge related to the use of the first two pain scales is that some questions are not applicable to cats with oral pain. For example, cats with oral pain often do not pay attention to the surgical area and it is often difficult to palpate a painful area (i.e., inside the oral cavity), which would be key behaviors in cats with other sources of pain including the abdomen and limbs (article 1). Thus, oral pain could be underestimated resulting in delays for analgesic intervention.

The Feline Grimace Scale (FGS) has been recently published and it comprises five action units (AU): eyes, ears, muzzle, whiskers, and head position. The instrument was developed and validated for naturally occurring pain of different sources and intensities (123). The clinical applicability of the FGS has been confirmed by comparing image with real-time assessment. In brief, minimal bias and narrow limits of agreement were observed between both methods of assessment (248). However, the FGS has not been specifically tested for assessment of oral pain, yet. In the authors' experience, multiple dental extractions can lead to facial edema which might influence the FGS scores. Therefore, there is an interest to understand the application and reliability of the FGS in cats undergoing oral treatment including dental extractions.

Pain assessment in the clinical setting requires real-time evaluation for early analgesic intervention. In laboratory animals (i.e., mice and rats), it is known that the presence of male evaluators affects pain scores, producing stress-induced pain inhibition (249). It is not known if a similar phenomenon also happens in cats.

The objectives of this study were to evaluate the inter-rater reliability of the FGS after oral treatment and the effect of the caregiver's presence on FGS scores. Our hypotheses were that the scores from different raters would be reliable and the presence of the caregiver would decrease the FGS score.

Materials and methods

Study design

Data for this study were obtained from a previously reported clinical trial involving dentistry, nutrition, pain management and behavior in cats before and after dental extractions (articles 1,2). The study was approved by the Institutional Animal Care and Use Committee of the Université de Montréal (protocol 17- Rech-1890) and performed at the Centre hospitalier universitaire vétérinaire (CHUV), Faculty of Veterinary Medicine, Université de Montréal, between July 2017 and February 2018. The study is reported according to the CONSORT guidelines (http://www.consort-statement.org). The study design was a prospective, blinded, randomized clinical trial.

Animals

Twenty-four healthy cats (6 ± 3.3 years old; 4.9 ± 1.7 kg, 11 and 13 neutered males and females, respectively) with or without naturally occurring oral disease were included. Cats were considered healthy based on history, medical records, physical examination, complete blood count and biochemical panel. Recruitment of cats from shelter facilities was performed by two investigators (PS and BM) after informed written consent. All cats were admitted the day before dental procedures (day 0), and they underwent dental treatment under general anesthesia on day 1 and were discharged on day 6. They were housed in stainless steel cages in a cat-only ward and had free access to water, litter box and bedding. The amount of food offered was calculated based on caloric requirement as previously reported (article 1).

Inclusion and exclusion criteria

Cats were divided in one of two groups according to the severity of oral disease: no/minimal oral disease (n = 12) or severe oral disease requiring dental treatment (n = 12) (article 1). Diagnostic and treatments including dental examination (evaluation of gingival and calculus index, periodontal disease staging, and the number of missing tooth and tooth resorption), radiography, scaling, polishing, and/or extractions were performed as needed. Enrollment into either no/minimal or severe oral disease group in each cat was determined after dental treatment based on the size and number of extracted teeth (article 1). Cats with a body condition score of < 3 or more than seven out of nine were not included. Cats with fearful behaviors, concurrent medical conditions, systemic disease, and the use of analgesics and/or antibiotics within a period up to 10 days before presentation were also not included.

Anesthesia, analgesia and dental treatment

Detailed description of anesthetic and monitoring procedures is available elsewhere (article 1). Briefly, premedication included the intramuscular (IM) administration of acepromazine (0.02 mg/kg; 1 mg/mL, Acepromazine maleate, Gentès & Bolduc, Saint-Hyacinthe, QC, Canada) and hydromorphone (0.1 mg/kg; 2 mg/mL, Hydromorphone hydrochloride, Sandoz, Boucherville, QC, Canada). Anesthesia was induced with intravenous (IV) propofol (10 mg/mL, Propoflo 28, Zoetis, Kirkland, QC, Canada) and maintained with isoflurane (Isoflurane USP, Fresenius Kabi, Toronto, ON, Canada) in oxygen. Under general anesthesia, complete dental examination, radiography, scaling/polishing and tooth extractions (if needed) were performed by a board-certified individual and a 3rd-year resident of the American Veterinary Dental College. Cats requiring tooth extraction received local anesthetic blocks with bupivacaine (5 mg/mL, Sensorcaine, AstraZeneca, ON, Canada) using a 1mL syringe and a 25-G needle (up to a total of2 mg/kg) as needed including infraorbital, maxillary, and/or inferior alveolar mandibular nerve blocks ~20min before extractions. At the end of dental treatment, all cats received meloxicam (0.2 mg/kg; Metacam 5 mg/mL Solution for Injection; Boehringer Ingelheim, Burlington, ON, Canada) subcutaneously. Oral administration of meloxicam

(0.05 mg/kg, Metacam 0.5 mg/mL Oral Suspension for Cats; Boehringer Ingelheim, Burlington, ON, Canada) was continued at 24, 48, and 72 h after the first dose according to label recommendations in Canada.

Real-time pain assessment, video recording and video editing

Real-time pain assessment was performed by one male observer [RW] using the CMPS-F at 23 different time-points from day 0 to 6. This observer was unaware of the oral condition and/or treatment of the cat. Video recordings were performed at 9 different time-points from day 0 to 6 for the study of orofacial pain-related behaviors using a wide-angle lens camera (GoPro Hero 5, GoPro, Riverside, CA, USA) set between the cage bars and remotely controlled by a smartphone (iPhone7, Apple Inc, Cupertino, CA, USA) (article 2). Cats were moved to a specific cage for video recording that included better lighting. After a 5-min acclimation to the new cage, 10-min videos were recorded for assessment of general (without the observer in the ward), playing, feeding and post-feeding behaviors (with the observer in room) for the purpose of studying different aspects of oral pain-induced pain behaviors (article 2). Briefly, the recordings of general and playing behavior were aimed to observe behaviors without interaction with the observer and the behaviors during playing with the observer using a ribbon toy, respectively. Data from selected time-points in which both real-time pain assessment and video recording had been performed were used in this study. These included the following four time-points: at 6 h postoperatively on day 1, at 8 am on day 6 and those recorded before and after rescue analgesia. These time points were chosen to represent a wide range of images of painful and non-painful cats. Video editing (trimming) was performed by the same observer [RW] using a video player software (QuickTime Player 10.5, Apple Inc, Cupertino, CA, USA) to obtain videos without the presence of the caregiver during recordings of general behaviors, and videos with the presence of the caregiver during recordings of playing behaviors. For the latter case, only recordings performed when the caregiver had entered the room but before playing with the cat using a ribbon toy were used.

During real-time pain assessment, if a cat had CMPS-F scores ≥ 5/20, rescue analgesia was administered with hydromorphone [0.05 mg/kg IV, if the IV catheter was in place (i.e.,

first 24 h after surgery) or 0.1 mg/kg IM, if the IV catheter had been removed]. CMPS-F scores were re-assessed 30min after rescue analgesia. Additional 5-min videos were recorded immediately before rescue analgesia and 30min after the administration of hydromorphone without the caregiver in the room.

Image collection

Following video editing (trimming), a total of 124 videos were randomized using a random permutation generator (http://www.randomization.com) and renamed to consecutive numbers. Image capture (i.e., screenshots) of cat faces was performed for each video by a different investigator [GD] who was not involved with image scoring. Screenshots were performed when the cat was facing the camera and the entire face was visible. Then, the screenshot that was considered the most representative on the entire video for that timepoint was selected. Images were not captured if the cat did not face the camera at any time during the video (no frontal image). Quality assessment of each screenshot was performed by the same individual who edited the videos [RW]. Image quality was assessed based on the angle of the face, brightness, blur, and whether the entire face including ear tips, whiskers and part of the proximal scapula were visible (Figure 1).

Figure 1. Flowchart of images captured from 24 cats with oral disease included in the study. Images with (a) and (b) were included for the analyses of inter-rater reliability and the effect of caregiver's presence, respectively

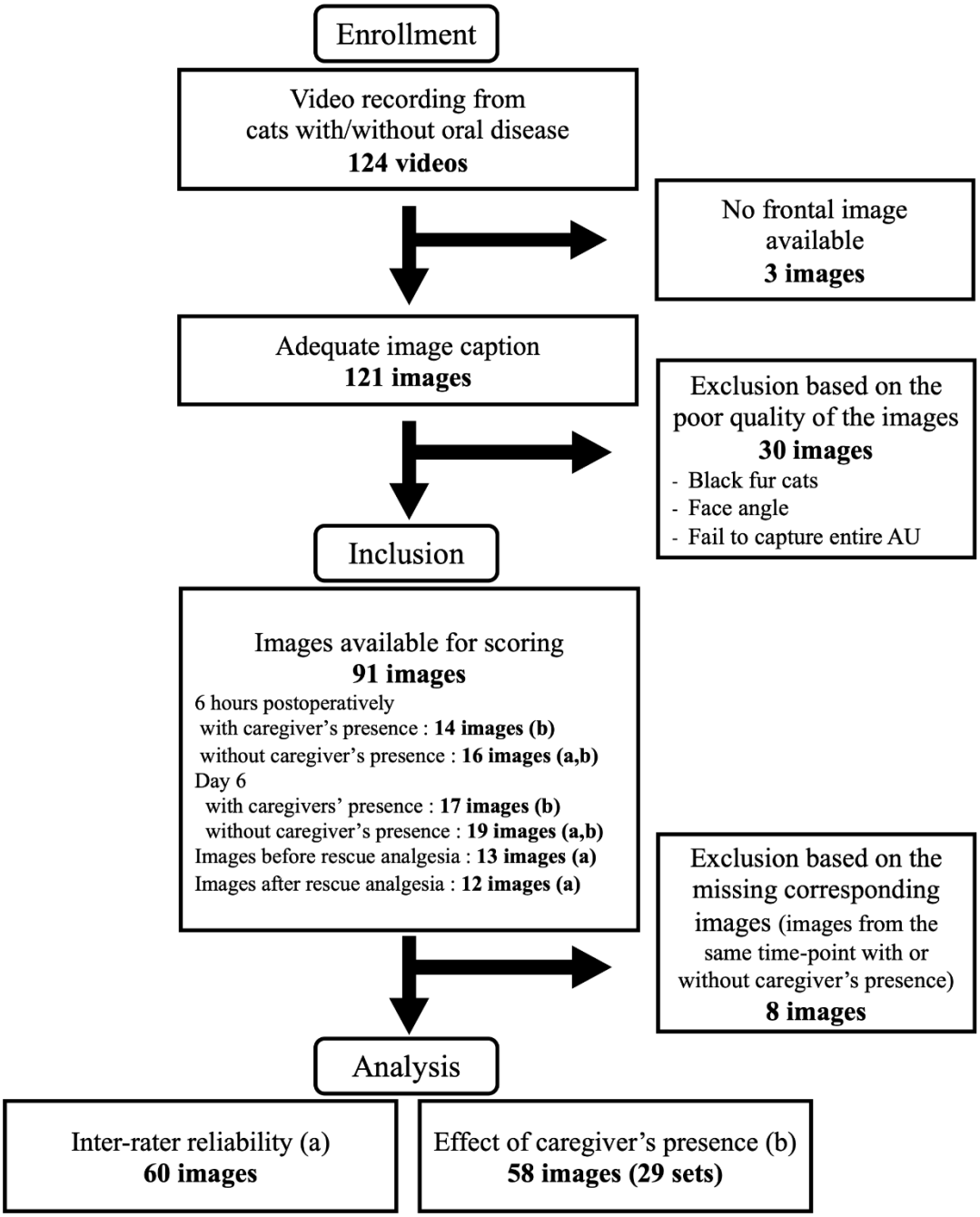

Image scoring

A total of 91 images were independently scored by 4 raters [ME, BM, HR, PS, three Ph.D. candidates (female) and one board-certified veterinary anesthesiologist (male)] who were blinded to the oral conditions of cats and timing of the recording (Figure 1). The raters were supplied with the training manual published with the original article (123) (https://static-content.springer.com/esm/art%3A10.1038%2Fs41598-019-55693-8/MediaObjects/41598_2019_55693_MOESM1_ESM.pdf). Each image was evaluated using the FGS for scoring of five action units (AU): ears, eyes, muzzle, whiskers, and head position. The AUs were scored as following: 0 = AU is absent; 1 = moderate appearance of the AU, or uncertainty over its presence or absence; 2 = obvious appearance of the AU; or "not possible to score" = e.g., if the AU was not clearly visible (123). A total score was calculated by the sum of the scores of the AUs divided by the total possible score, excluding those marked as not possible to score (e.g., 3/8 = 0.375). The images were scored using an online survey (SurveyMonkey, https://www.surveymonkey.com) and divided into two sets. There was a minimum of 24 h and maximum of 48 h between scoring of the first and second set of images to avoid rater's fatigue. Scoring was performed between May 21st and 24th, 2019. Images receiving "not possible to score" for two or more AUs were excluded from statistical analyses.

Statistical analyses

Statistical analyses were performed using SPSS software (version 25.0 IBM SPSS Statistics, Armonk, NY, USA). Images from days 1 and 6 without the caregiver's presence and images before and after rescue analgesia were used for the analysis of inter-rater reliability. Images from days 1 and 6 with and without caregiver's presence were used for the analysis of effect of caregiver. Inter- rater reliability was calculated for each AU and for the total FGS score using intraclass correlation coefficients (ICC) with 2-way random effects ICC model for absolute agreement. ICC was interpreted according to a previously described scale (250): < 0.5 = poor, 0.5–0.75 = moderate, 0.75–0.9 = good, and > 0.90 = excellent reliability. The ICC was calculated based on single measures (ICC_{single}) which is an index for the reliability of the rating for one rater and the average of the measures

($ICC_{average}$) which is an index for the reliability of mean of k raters as recommendation of the guideline (250). The effect of the caregiver's presence was assessed by comparing FGS scores of images with and without caregiver's presence using a Wilcoxon signed rank test. The FGS scores with and without the caregiver's presence were compared between no/minimal and severe oral disease cats using a Mann-Whitney U-test, and within each group using a Wilcoxon signed rank test. Normality of the distribution of the scores was assessed using a Shapiro-Wilk test. Values of $p < 0.05$ were considered statistically significant.

Results

Inter-rater reliability

Sixty images without the caregiver's presence were included in the analysis. Images were available from days 1 and 6 (n = 16 and n = 19, respectively) and from before and after rescue analgesia from days 1, 2 and 3 (n = 13 and n =12, respectively) (Figure 1). Inter-rater reliability is presented in Table 1. ICC_{single} was moderate for ears, muzzle, whiskers, and head position and good for eyes. The $ICC_{average}$ was good for muzzle and excellent for ears, eyes, whiskers and head position. Reliability of total FGS scores was good and excellent, based on ICC_{single} and $ICC_{average}$, respectively.

Table 1. Inter-rater reliability of the Feline Grimace Scale in cats with oral disease

Action unit		ICC (95% CI)
Ears	ICC_{single}	0.68 (0.55 - 0.78)
	$ICC_{average}$	0.89 (0.83 - 0.94)
Eyes	ICC_{single}	0.76 (0.65 - 0.84)
	$ICC_{average}$	0.93 (0.88 - 0.95)
Muzzle	ICC_{single}	0.56 (0.43 - 0.69)
	$ICC_{average}$	0.84 (0.75 - 0.90)
Whiskers	ICC_{single}	0.64 (0.50 - 0.76)
	$ICC_{average}$	0.88 (0.80 - 0.93)
Head position	ICC_{single}	0.74 (0.63 - 0.82)
	$ICC_{average}$	0.92 (0.87 - 0.95)
FGS total score	ICC_{single}	0.84 (0.77 - 0.89)
	$ICC_{average}$	0.95 (0.93 - 0.97)

A total of 91 images were independently scored by 4 raters who were blinded to the oral conditions of cats and timing of the recording. Intraclass correlation coefficient (ICC) estimates with 95% confidence intervals (95% CI) were calculated based on single measures (ICC_{single}) and average ($ICC_{average}$) of measures, using a 2-way random effects model for absolute agreement. Interpretation of ICC was performed as following: ICC < 0.5 = poor, 0.5-0.75 = moderate, 0.75-0.9 = good, and > 0.90 = excellent reliability.

Effect of caregiver's presence

A total of 66 images were collected. From these, 29 images (13 and 16 sets from male and female cats, respectively) had a corresponding match (i.e., image from the same time-point with or without caregiver's presence), resulting in 58 images to be scored (day 1, n = 28 and day 6, n = 30). A total of 8 images did not have the corresponding match and were excluded (Figure 1). Median (range) of total FGS score without and with caregiver's presence were 0.088 (0–0.525) and 0.075 (0–0.325), respectively. Overall, there were not

significant differences between scores with and without the caregiver's presence (p = 0.12). Median (range) of FGS scores without the caregiver's presence was 0.088 (0–0.325) in the minimal and 0.088 (0–0.525) in the severe group (p = 1.000). Median (range) FGS scores with the caregiver's presence in each group was 0.075 (0–0.325) in the minimal and 0.063 (0–0.250) in the severe group (p = 0.711). The FGS scores were not significantly different with or without the caregiver's presence within the no/minimal group (p = 0.195) or severe group (p = 0.398).

Discussion

This study evaluated the inter-rater reliability of the FGS for pain assessment in cats with naturally occurring oral disease and the effect of the caregiver's presence on FGS scores. Overall, the results indicate that the reliability of each AU and total FGS scores based on ICC_{single} were moderate to good and that the presence of a male caregiver had no significant effect on the FGS scores.

Inter-rater reliability of total FGS scores was good to excellent considering ICC_{single} and $ICC_{average}$. The estimate ICC_{single} is commonly used when a decision is made based on the scores of a single rater, however values of $ICC_{average}$ are usually higher (250). In the current study, the inter-rater reliability for each AU was moderate (ears, muzzle, whiskers, and head position) to good (eyes). Reliability of scores of the muzzle and whiskers were lower than other AUs (ICC_{single} for muzzle and whiskers were 0.56 and 0.64, respectively). It is possible that dental extractions caused inflammation and facial edema likely impacting the scoring of muzzle and whiskers (i.e., difficulty of distinction between postoperative inflammation and the painful facial expression). Nevertheless, similar results were observed in the previous study in cats (0.63 and 0.55 for the muzzle and whiskers, respectively) (123). Reliability of the AUs ears and head position (0.68 and 0.74, respectively) were lower than the previous study (0.87 and 0.90, respectively) (123). In the present study, the camera was positioned to film the cats' behaviors for another study (article 2), and the height and angle of the video camera (set higher in the cage) may have not been ideal to capture the frontal image of the cat and further FGS scoring. If the camera angle is not optimal, the visualization, and interpretation of AUs could change

between raters. However, ICC_{single} of total scores were good, and the result indicates that the raters could still identify the changes associated to pain in these cats.

In this study, 51.7% (15/29) of images with the caregiver's presence had lower, yet not significant, scores than those without caregiver's presence. Indeed, the caregiver's presence did not significantly affect the FGS scores either when data for each group were analyzed together or independently. On the other hand, a previous study reported that the presence of a male experimenter produced a stress-induced pain inhibition response in mice and rats (249). This previous study reported that this response disappears within 30–60min and it is not known if longer acclimation periods would change the FGS scores with or without the presence of a caregiver in cats. Furthermore, in the present study, a male observer scored male and female cats during the study whereas male and female raters scored the images. The sex of the observer is known to affect real- time pain assessment in rodents (i.e., male pheromone induces analgesic effect) (249); similar findings have been reported with video-assessment in small animals (251). Although the present study was not specifically designed to evaluate the effect of sex on pain assessment, the presence of a male caregiver did not affect FGS scores via image assessment. However, it is not known if the sex of raters could have influenced FGS scores.

There are some limitations in this study. First, this was an exploratory study and the materials were obtained from previous reports (articles 1,2). As a result, sub-optimal image quality played an important role as discussed above. Indeed, 26.6% of the images were excluded. Additionally, power analysis and sample size calculation were not performed before the experiment because there is no consensus to determine the sample size a priori in the validation studies (252). Second, the order of video recording could not be randomized, and the videos without the caregiver's presence were always obtained before those with the caregiver's presence. However, this order bias was not present during image assessment because the videos were trimmed, and images were randomized before image selection and scoring by an observer not involved with image scoring. Third, images of cats presenting moderate (nine images) to severe (13 images) pain based on CMPS-F (3-4, and ≥ 5, respectively) were underrepresented. This could

represent an important limitation to study the effects of caregiver's presence. If the images of painful cats were underrepresented, it is possible that some of these patients had low FGS scores which could not be significantly reduced during a stress-induced pain inhibition response with the caregiver's presence, as observed previously (249). One of the reasons for the lack of good quality images was that three black cats required rescue analgesia, and five of these images were excluded from analysis because identification of muzzle and whiskers were not possible in these individuals. This issue was also reported in previous studies in horses and cats (123,253), and a possible solution would be the use of artificial lighting sources during recordings. The other possible way to balance the distribution of pain intensity across the images might be to obtain several screenshots from same painful time points (i.e., videos filmed before rescue analgesia). However, the increase of number of images from same cats could bias the raters' scores. Finally, images of days 1 and 6 were included for the analysis of the effect of caregiver's presence. The images obtained on day 6 might have biased the results since perhaps cats were no longer painful. However, the pain scores (CMPS-F) in the severe group were significantly higher than the minimal group on day 6 (article 1), which made the authors believe cats in severe group could still be in mild pain.

In conclusion, the FGS is a reliable tool for assessment of oral pain in cats, though some action units were difficult to identify due to poor image quality and facial edema and inflammation. The caregiver's presence did not affect the FGS scores. The influence of sex in the FGS scores should be a subject of future investigations.

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Article 4: The analgesic effects of buprenorphine (Vetergesic or Simbadol) in cats undergoing dental extractions: A randomized, blinded, clinical trial

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Authors' contributions

Conceptualization: RW, PSV.

Data curation: RW, JM, MEC, PSV.

Formal analysis: RW, PSV.

Funding acquisition: PSV.

Investigation: RW, JM, MEC, YD, PSV.

Methodology: RW, PSV.

Project administration: PSV.

Resources: PSV.

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Abstract

This study aimed to evaluate the analgesic efficacy of two dosage regimens using two different concentrations of buprenorphine in cats undergoing dental extractions. Twenty-three cats with oral disease (8.2 ± 2.2 years old; 4.9 ± 0.9 kg) were included in a prospective, blinded, randomized clinical trial. Cats randomly received either Simbadol (1.8 mg/mL; 0.24 mg/kg, subcutaneously, every 24h: SG, n = 11) or Vetergesic (0.3 mg/mL; 0.02 mg/kg, intra-muscularly, every 8h: VG, n = 12) throughout the study. They were admitted at day 0, underwent oral examination/radiographs/treatment under general anesthesia (buprenorphine-propofol-isoflurane-meloxicam-local anesthetic blocks) at day 1 and discharged at day 4. Sedation and pain were scored using the dynamic interactive visual analog scale (day 1) and the Glasgow Composite Measure Pain Scale-Feline (CMPS-F; up to postoperative 8 hours at day 1, 8 am, 4 pm and midnight at days 2 and 3, and 8 am at day 4), respectively. Rescue analgesia was administered with hydromorphone (0.05 mg/kg intravenously on day 1 or 0.1 mg/kg intramuscularly after day 2) when CMPS-F ≥ 5. Resentment defined as any type of escape behavior associated with aversion to drug administration was recorded. Sedation and pain scores, the prevalence of rescue analgesia and resentment during drug administration were analyzed using linear mixed models and Fisher's exact test, respectively ($p < 0.05$). Pain and sedation scores were not significantly different between groups. Sedation scores were significantly higher up to postoperative 2 hours in both groups. Pain scores in SG and VG were significantly higher up to postoperative 8 hours and 8 am of day 2, respectively, than baseline. Prevalence of rescue analgesia and resentment were not significantly different between groups (SG: 27.3%, VG: 33.3% and SG: 0%, VG: 25%, respectively). Simbadol produced similar analgesic effects to Vetergesic without resentment during drug administration.

Introduction

Periodontal disease including gingivitis and periodontitis is a plaque-induced pathology and is a serious health problem. It produces pain and inflammation and decrease food intake in both human and companion animals (41,177,178, article 1). In cats, multiple dental extractions are commonly required for treatment and the procedure can be invasive and painful. Studies in our laboratory showed that cats require long-term analgesic treatment with opioids, local anesthetic blocks and nonsteroidal anti-inflammatory drugs (NSAIDs) after multiple dental extractions (article 1). Opioid analgesics are commonly administered as part of perioperative multimodal analgesia for acute pain management in veterinary medicine (163). However, full agonists of μ-opioid receptors like hydromorphone, oxymorphone and fentanyl are not approved for use in companion animals. Additionally, their unavailability in North America is becoming a critical issue that can jeopardize animal care and welfare.

Buprenorphine is a potent highly lipophilic analgesic opioid that is largely used in the treatment of acute pain. The drug is generally considered as a partial agonist of μ opioid receptors. Buprenorphine is often administered to treat pain in cats as adverse effects have been rarely reported. Cats usually display euphoric behavior and buprenorphine has shown to produce mechanical and thermal antinociceptive effects (139,161,162). On the other hand, the drug has failed to provide analgesia in some cats undergoing ovariohysterectomy (167). For this reason, the drug is commonly administered as part of multimodal analgesia. Indeed, the prevalence of analgesic failure is lower when buprenorphine is administered in combination with other analgesics than alone (139,210,254).

Vetergesic™ (buprenorphine hydrochloride injection, 0.3 mg/mL, Champion Alstoe, Whitby, ON, Canada) is approved for use in cats in several countries. For example in Canada, the labeled dose for intramuscular administration of Vetergesic is 0.02 mg/kg. Indeed, this concentration is similar to formulations of buprenorphine used in humans that are often administered “off-label” in veterinary medicine (e.g. Buprenex™). Simbadol™ (buprenorphine hydrochloride injection, 1.8 mg/mL, Zoetis, Parsippany, New Jersey, USA) is an FDA-approved opioid analgesic for cats. The medication package insert

indicates that Simbadol provides 24-hour pain control after a single dose subcutaneously; a total of three injections can be administered for postoperative analgesia. Due to its long-lasting analgesic properties and FDA approval for use in cats, there is an interest in administering buprenorphine for the treatment of pain associated with dental extractions in combination with dental nerve blocks and the administration of NSAIDs in this species. Additionally, it is not known if single or multiple daily injections of Simbadol or Vetergesic using different routes and intervals of administration, respectively, would produce different analgesic effects and frequency of adverse events (i.e. resentment to drug administration). It could be possible that different dosage regimens could still yield similar analgesic effects.

The objective of the study was to evaluate the analgesic efficacy and adverse events of Simbadol in comparison with Vetergesic as part of a multimodal regimen in cats undergoing dental extractions. Our hypothesis was that the two treatments would produce similar postoperative pain scores, adverse events and timing and prevalence of rescue analgesia when using the Glasgow Composite Measure Pain Scale-Feline (CMPS-F) (121).

Materials and methods

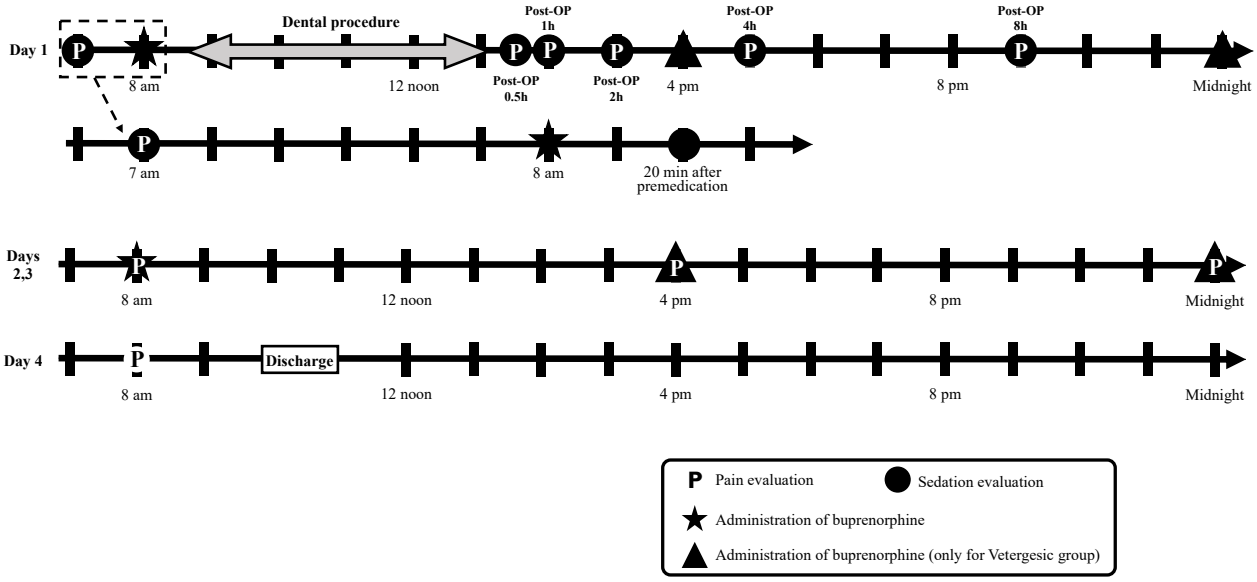
Study design

The study design was a prospective, blinded, randomized clinical trial. All experimental procedures were approved by the institutional animal care and use committee of the Université de Montréal (18-Rech-1927) and this study is reported according to the CONSORT guidelines [CONSORT guideline; http://www.consort-statement.org]. The experimental study was performed at the Centre hospitalier universitaire vétérinaire (CHUV), the veterinary teaching hospital of the Faculty of Veterinary Medicine of the Université de Montréal, from August 2018 to April 2019.

Animals

Thirty adult client-owned cats were recruited after informed written consent. Cats were included based on medical records, complete physical examination, and hematology and biochemical panel and had to be free of systemic disease. Cats with body condition score between 3-7 out of 9, and with moderate to severe oral disease were included. Disease severity was determined using a dental scoring system which involved the number and location of teeth extraction: canine tooth: 3 points, third premolar of maxilla or molar of mandible: 2 points, second premolar of maxilla or premolar of mandible: 1 point. A score of 2 points was given if seven or more incisive teeth and/or first premolars of the mandible were extracted; a score of 1 point was given if six or fewer teeth were extracted (article 1). The total dental score was calculated and cats with dental score ≥ 6 were included in this study. Cats were excluded if they presented fearful behavior that could impair pain assessment, concurrent medical conditions or diseases (i.e. cancer, renal, cardiovascular, hepatic, or gastrointestinal disease) and/or received any medication including analgesics and antibiotics for up to 10 days before the study had begun. Cats were admitted at day 0 and underwent oral examination, radiographs and treatment under general anesthesia at day 1 (Figure 1). All patients were discharged at day 4.

Figure 1. Schematic of time points for administration of buprenorphine during the study. The timeline demonstrates and example of a 4-hour dental procedure in a cat including time points of pain and sedation assessment.



Group allocation

All cats were randomly allocated into one of two treatments groups: Vetergesic group [Vetergesic 0.02 mg/kg intramuscularly (IM) three times a day (8 am, 4 pm and midnight) for 3 days] or Simbadol group [Simbadol 0.24 mg/kg subcutaneously (SC) once a day (8 am) for 3 days]. Randomization was performed using a random permutation generator (http://www.randomization.com) (Figure 1). IM and SC administration were always performed over the epaxial muscles and between the shoulder blades, respectively, by individuals not involved with sedation or pain assessment (ME and PS).

Anesthetic and surgical procedures

All cats were premedicated with either Vetergesic or Simbadol at the doses described above. A eutectic mixture of local anesthetic cream (EMLA cream lidocaine 2.5% and procaine 2.5% cream, Astra Zeneca, Mississauga, ON, Canada) was applied and covered with plastic film and adhesive bandage after clipping the hair over the skin of one of the

cephalic veins. A 22-G x 1-inch needle intravenous (IV) catheter was aseptically placed in the cephalic vein approximately 20 minutes after premedication. Anesthesia was induced with propofol (Propoflo 28, 10 mg/mL, Zoetis, Kirkland, QC, Canada) administered IV to allow endotracheal intubation after spraying the arytenoid cartilages with 0.05 mL of lidocaine 2% (Lidocaine hydrochloride sterile injection, 20 mg/mL, Vétoquinol N.-A.Inc, Lavaltrie, QC, Canada). The endotracheal tube was then connected to a coaxial Mapleson D system. Anesthesia was maintained with isoflurane vaporized in oxygen by a single veterinarian with experience in anesthesia (ME). Hemoglobin oxygen saturation, heart rate obtained from a lead II electrocardiography, respiratory rate, end-tidal carbon dioxide, inspired and expired concentrations of isoflurane, indirect blood pressure via oscillometry, and rectal temperature were monitored every 5 minutes during anesthesia using a multiparametric monitor (Lifewindow 6000V Veterinary Multiparameter Monitor; Digicare Animal Health, Boynton Beach, FL, USA). Blood pressure was also monitored with a Doppler flow monitor and a sphygmomanometer. The cuff width used for blood pressure monitoring was approximately 40% of the limb circumference. Lactated Ringer's solution (Lactated Ringer's Inj. Bag / 500 mL, McCarthy & Sons Service, Calgary, AB, Canada) was administered at 5 mL/kg/hour during the first hour of the procedure. Fluid rates were then adjusted based on the cat's hydration status and requirements (2-5 mL/kg/hour). If hypotension was observed (mean arterial blood pressure < 60 mmHg), a bolus of the isotonic solution (5 mL/kg over 15 minutes) was given. Dental nerve blocks including the infraorbital, maxillary and/or inferior alveolar mandibular nerve blocks were performed with bupivacaine 0.5% (Sensorcaine, 5 mg/mL, AstraZeneca, ON, Canada) using a 25-G needle based on the location of dental extractions (0.2–0.3 mL/site depending on the number of blocks required after radiographs and approximately 20 minutes before the procedure). The block was repeated if the sympathetic responses to surgical stimulation were observed during dental extractions. The total dose of bupivacaine for all anesthetic blocks did not exceed 2 mg/kg. Meloxicam (0.2 mg/kg, SC, Metacam 5 mg/mL Solution for Injection; Boehringer Ingelheim, Burlington, ON, Canada) was administered at the end of the surgical procedure. Oral administration of meloxicam (0.05 mg/kg, Metacam 0.5 mg/mL Oral Suspension for Cats; Boehringer Ingelheim, Burlington, ON, Canada) were continued for three days at 24, 48 and 72 hours after the

first dose according to the label recommendations in Canada. Dental treatment was performed by a resident (JM) and a board-certified veterinarian (YD) of the American Veterinary Dental College (AVDC). Dental parameters [i.e. periodontal disease staging (0–4), gingival, calculus and plaque index (0–2), number of teeth extraction and dental score] were evaluated under general anesthesia (230-232). Anesthesia time (time elapsed from induction of propofol to turning off the vaporizer dial of isoflurane), procedure time (time elapsed from start of dental procedure [i.e. dental scaling] to end of all procedures [i.e. polishing]) and surgery time (time elapsed from the first incision until placement of the last suture) were recorded.

Sedation scores

Sedation scores were evaluated by an individual (RW) who was unaware of treatment groups using the dynamic and interactive visual analog scale (DIVAS) where 0 was considered as no sedation and 100 as maximum sedation (255). These evaluations were performed approximately 60 min prior to the premedication (baseline), 20 min after premedication, and at 0.5, 1, 2, 4, 8 hours postoperatively at day 1 (Figure 1).

Pain scores

The CMPS-F (121) and Feline Grimace Scale (FGS) (256) were used to evaluate pain. Data regarding the FGS are not presented here and will be used as part of additional validation of the tool in cats undergoing dental extractions. The outcome of this study was solely based on the CMPS-F scores. Pain was always assessed by the same individual who also evaluated sedation. Pain scoring was performed at the same time points described above for sedation at day 1 (with the exception of 20 min after premedication), and at 8 am, 4 pm and midnight on days 2 and 3, and at 8 am on day 4 (Figure 1).

Resentment to drug administration

Resentment was considered any type of escape behavior associated with aversion to drug administration including vocalization, hissing, growling and attempt to bite. Resentment was recorded as present or absent by the individuals who administered buprenorphine during drug administration.

Rescue analgesia

Cats were administered hydromorphone either at 0.05 mg/kg IV (if the intravenous catheter was in place, at day 1) or 0.1 mg/kg IM (if the intravenous catheter had been removed, at days 2 to 4) if CMPS-F scores were ≥ 5/20. Pain assessment was performed 30 minutes after rescue analgesia to ensure the patient's comfort. Pain and sedation scores obtained after rescue analgesia were excluded from the statistical analysis, but assessments of sedation and pain were continued until the end of the study. Treatments with buprenorphine were stopped after the administration of hydromorphone.

Statistical analyses

Statistical analyses were performed using standard statistical software (SPSS Statistics V25, IBM, USA). Power analysis was calculated before the study and indicated that a sample size of 8 cats per group would be required to detect a difference of 3 points between the two groups using the CMPS-F with an alpha value of0.05, a power of 80% and a standard deviation of 2 points. The sample size was increased to compensate for any individual variability in pain scores and the potential for cats with dental scores < 6 that would lead to patient exclusion. Data were tested for normality using a Shapiro-Wilk test. Demographic data for each treatment group were compared using independent t-test or Mann-Whitney U test where appropriate. To normalize the distribution of sedation scores, $\log_{10}$ transformation was performed after adding one to all values because baseline values were zero. Sedation and pain scores were compared between treatments and between baseline and each time point using a linear mixed model for repeated measures. Time and treatment group, and their interaction were considered as fixed

effects. Cat was considered a random effect and dental score was added as a covariate to the model. The best structures of the covariance (first order autoregressive) were assessed using information criteria that measured the relative fit of a competing covariance model. The Benjamini-Hochberg procedure was used to adjust the alpha level for each comparison. The prevalence of rescue analgesia and resentment (dichotomized data) during administration of buprenorphine were compared between treatment groups using Fisher's exact test. Values of $p < 0.05$ were considered statistically significant.

Results

Seven cats were excluded from the study; six cats were excluded because of dental scores < 6 and one cat developed fearful behavior during hospitalization after dental treatment. Therefore, 23 cats were included (12 cats in Vetergesic group and 11 cats in Simbadol group). The local anesthetic block was repeated in twelve cats (6 cats in each group). Temporary mild hypotension was observed in twelve cats (6 cats in each group) which improved after the fluid bolus.

One cat in Simbadol group developed upper respiratory disease and conjunctivitis in the evening of day 3. Antibiotics [amoxicillin/clavulanic acid (125 mg/kg PO BID, Clavamox, Zoetis, Kirkland, QC, Canada) and tetracycline (eye lube TID, Terramycin, Zoetis, Kirkland, QC, Canada)] were administered for 10 days. One cat in Vetergesic group developed asthma and upper respiratory disease at day 2 (i.e. noon) which required antibiotics (amoxicillin/clavulanic acid: 62.5 mg/kg PO BID for 14 days) and inhalation administration of fluticasone (250 µg BID, Flovent HFA, GlaxoSmithKline Inc., Mississauga, ON) and salbutamol 100 µg/spray BID, Ventolin HFA, GlaxoSmithKline Inc., Mississauga, ON). These two cats were discharged without severe clinical signs. Data obtained after the development of clinical signs were excluded from the statistical analysis.

Demographic data and dental parameters

Breed and gender distribution are shown in Table 1. Demographic data, propofol requirements, and anesthesia, procedure and surgery times are shown in Table 2. Dental parameters are shown in Table 3. There were not significant differences between groups for the information presented in Tables 2 and 3.

Table 1. Demographic data including gender, reproductive status and breed of cats undergoing dental extractions and treated with Simbadol or Vetergesic

	Category	Simbadol (n = 11)	Vetergesic (n = 12)
Gender	**Neutered male**	8	5
	Spayed female	3	7
Breed	**Domestic short hair**	8	8
	Domestic long hair	3	4

Table 2. Demographic data including age, body weight, body condition score, propofol requirements for anesthetic induction, and anesthesia, procedure and surgery times. Values are expressed as mean ± SD except for body condition score which is reported as median (range)

Variable	Simbadol (n = 11)	Vetergesic (n = 12)	p value
Age (years)	7.9 ± 2.2	8.5 ± 2.3	0.535
Body weight (kg)	5.2 ± 0.8	4.6 ± 0.9	0.154
Body condition score (1-9)	5 (5-7)	5 (5-7)	0.260
Propofol requirements (mg/kg)	4.9 ± 1.3	5.0 ± 1.1	0.582
Anesthesia time (min)	283.6 ± 88.7	313.8 ± 81.0	0.402
Procedure time (min)	268.2 ± 89.5	298.3 ± 83.5	0.413
Surgery time (min)	210.2 ± 83.7	232.7 ± 86.6	0.534

Table 3. Dental parameters including periodontal disease staging, gingival, calculus and plaque index, number of tooth extractions and dental score. Values are expressed as median (range)

Parameter	Simbadol (n = 11)	Vetergesic (n = 12)	p value
Periodontal disease staging (0-4)	3 (1-4)	3 (1-4)	0.658
Gingival index (0-3)	2 (1-3)	2 (1-3)	0.786
Calculus index (0-3)	2 (0-3)	2 (1-3)	0.326
Plaque index (0-3)	2 (1-3)	2 (1-3)	0.379
Number of tooth extraction	11.5 (5-22)	18 (10-23)	0.328
Dental score (0-28)	10.5 (8-22)	15.5 (7-25)	0.356

Sedation scores

DIVAS scores are shown in Table 4. There were no differences between groups ($p > 0.160$, $df > 80.20$). In both groups, DIVAS scores after sedation and postoperative 0.5, 1 and 2 h were significantly higher than baseline.

Table 4. Dynamic and interactive visual analog scale (DIVAS) scores in cats undergoing dental extractions after the administration of Simbadol or Vetergesic. Values are expressed as median (range)

Time points	Groups	DIVAS	p value between groups	p value compared with baseline
Baseline	Simbadol (n = 11)	0 (0)	0.816	
	Vetergesic (n = 12)	0 (0)		
20 min after premedication	Simbadol (n = 11)	7 (0-9)	0.453	< 0.0001*
	Vetergesic (n = 12)	6.5 (0-14)		< 0.0001*
Postoperative 0.5 h	Simbadol (n = 11)	25 (3-57)	0.160	< 0.0001*
	Vetergesic (n = 12)	37 (17-92)		< 0.0001*
Postoperative 1 h	Simbadol (n = 11)	13 (5-41)	0.897	< 0.0001*
	Vetergesic (n = 12)	14.5 (0-86)		< 0.0001*
Postoperative 2h	Simbadol (n = 11)	6 (0-36)	0.483	0.0005*
	Vetergesic (n = 12)	2.5 (0-86)		0.0009*
Postoperative 4 h	Simbadol (n = 11)	0 (0-26)	0.879	0.097
	Vetergesic (n =11)	0 (0-74)		0.028
Postoperative 8 h	Simbadol (n = 9)	0 (0)	0.807	0.906
	Vetergesic (n = 10)	0 (0-13)		0.502

*Significant difference after adjustment

CMPS-F

CMPS-F scores are shown in Table 5. There were no significant differences between groups ($p > 0.148$, $df > 44.29$). In the Vetergesic group, CMPS-F scores were higher at 4 and 8 hours on day 1 and 8 am on day 2 compared with baseline. In the Simbadol group, CMPS-F scores were higher at postoperative 4 and 8 hours on day 1 compared with baseline ($p < 0.001$ in these time points).

Table 5. Pain scores using the Glasgow Composite Measure Pain Scale-Feline (CMPS-F) in cats undergoing dental extractions after the administration of Simbadol or Vetergesic. Values are expressed as mean (SEM).

	Time points	Treatments	CMPS-F	p value between groups	p value compared with baseline
Day 1	Baseline	Simbadol (n = 11)	0.7 (0.5)	0.858	
		Vetergesic (n = 12)	0.8 (0.4)		
	Postoperative 0.5 h	Simbadol (n = 11)	0.9 (0.5)	0.558	0.571
		Vetergesic (n = 12)	0.5 (0.4)		0.438
	Postoperative 1 h	Simbadol (n = 11)	1.5 (0.5)	0.148	0.068
		Vetergesic (n = 12)	0.6 (0.4)		0.676
	Postoperative 2 h	Simbadol (n = 11)	2.0 (0.5)	0.371	0.007
		Vetergesic (n = 12)	1.4 (0.4)		0.126
	Postoperative 4 h	Simbadol (n = 11)	2.5 (0.5)	0.920	0.0004*
		Vetergesic (n =11)	2.4 (0.4)		0.0006*
	Postoperative 8 h	Simbadol (n = 9)	2.6 (0.5)	0.759	0.0005*
		Vetergesic (n = 10)	2.8 (0.5)		< 0.0001*
Day 2	8 am	Simbadol (n =9)	2.3 (0.5)	0.234	0.004
		Vetergesic (n =9)	3.1 (0.5)		< 0.0001*
	4 pm	Simbadol (n =8)	1.7 (0.5)	0.775	0.058
		Vetergesic (n =7)	1.9 (0.5)		0.037
	12 pm	Simbadol (n =8)	1.4 (0.5)	0.883	0.168
		Vetergesic (n =7)	1.3 (0.5)		0.315
	8 am	Simbadol (n =8)	1.2 (0.5)	0.596	0.372
		Vetergesic (n =7)	1.5 (0.5)		0.177
Day 3	4 pm	Simbadol (n =8)	1.6 (0.5)	0.297	0.080
		Vetergesic (n =7)	0.9 (0.5)		0.796
	12 pm	Simbadol (n =7)	1.4 (0.5)	0.276	0.208
		Vetergesic (n =7)	0.6 (0.5)		0.772
Day 4	8 am	Simbadol (n =7)	0.6 (0.5)	0.861	0.925
		Vetergesic (n =7)	0.7 (0.5)		0.953

*Significant difference after adjustment.

Resentment to drug administration

Resentment was observed during the administration of buprenorphine in three cats in the Vetergesic group (3/12 cats; 25%; two cats at day 2 and one cat at day 3) and none of the cats in the Simbadol group (0/11; 0%) (p = 0.12).

Rescue analgesia

Rescue analgesia was administered to four cats in the Vetergesic group (4/12 cats; 33.3%), and three cats in the Simbadol group (3/11 cats; 27.3%) (Table 6). Prevalence of rescue analgesia was not different between groups (p = 0.56).

Table 6. Number of cats receiving rescue analgesia at each time point during the study

Group	Day 1 (postoperative)					Day 2	Days 3 and 4	Number of cats	Total dose	p value
	0.5 h	1 h	2 h	4 h	8 h					
Simbadol (n = 11)	0	0	0	2	0	1	0	3 (27.3%)	3	0.56
Vetergesic (n = 12)	0	0	1	1	1	3	0	4 (33.3%)	6	

Discussion

This study showed that Simbadol produced similar analgesic effects to Vetergesic without resentment during drug administration in cats with oral disease undergoing dental treatment. Pain score were not significantly different between treatments; however, pain scores were significantly increased longer in the Vetergesic group than Simbadol when compared with baseline. This result suggests that the analgesic effects of a single dose of Simbadol (subcutaneous administration of 0.24 mg/kg) could be long-lasting for dental extractions in cats in comparison with the dosage regimens used in the Vetergesic group

(intramuscular administration of 0.02 mg/kg every 8 hours). The dose of Vetergesic was based on label recommendations in Canada where the drug is used for postoperative pain relief at 0.01-0.02 mg/kg intramuscularly with an option to repeat a second dose two hours after the first injection, if necessary. Alternatively, the use of other classes of analgesics (i.e. multimodal analgesia) is also recommended in the label as it was done in this study with the combination of local anesthetics and NSAIDs. The frequency of administration for Vetergesic was determined based on the duration of analgesic effect for buprenorphine (135,257). Additionally, the study attempted to mimic intramuscular injections that would be used in clinical practice in the absence of an intravenous catheter. However, it is reasonable to argue that intravenous administration of buprenorphine could have produced more profound analgesia than the intramuscular route. It is also arguable that the analgesic effects of Vetergesic could have been more appropriate if injections were made every 6 hours, however that would have produced even greater prevalence of resentment during drug administration compromising feline welfare. Indeed, three cats in the Vetergesic group showed resentment and this difference would have been significantly different than Simbadol if one more cat in the Vetergesic group had had resentment. Since intramuscular injections are known to be painful (137), the frequency of injection should be minimized as much as possible for the ethical reasons (258). The buccal (transmucosal) route of administration could have also been considered in this study. However, it has failed to produce clinical analgesia after administration of buprenorphine in cats especially considering that the cats underwent a dental procedure and the presence of sutures and inflammation could preclude the use of the buccal route (247). Therefore, finally it was not considered an option for pain relief in this study. In feline practice, the administration of analgesics should be performed based on the patient's needs using pain scoring systems rather than a predetermined regimen (259). This is particularly true when considering the individual variability after the administration of intramuscular buprenorphine hydrochloride (139,161). For example, the duration of thermal antinociception was observed for only 60 minutes even considering a relative long elimination half-life of 460 ± 285 minutes (139). This gap is often explained by negative hysteresis where plasma concentrations of the drug does not correspond to analgesic efficacy. On the other hand, SC administration of Simbadol 0.24 mg/kg produced thermal

antinociception up to 24 hours (162). This should explain why pain scores returned to baseline values in the morning of day 2 in the Simbadol group. However, both treatments produced similar pain scores and prevalence of rescue analgesia.

There is a possible concern that multimodal analgesia may have biased our results. On the other hand, all cats received meloxicam and local anesthetic blocks with bupivacaine allowing the study design to compare Vetergesic and Simbadol when administered as part of multimodal analgesia. The administration of dental nerve blocks with bupivacaine might have influenced early postoperative pain scores since timing between the last dental nerve block and the end of anesthesia was approximately 1.5 hours. However, in both groups, some cats required early administration of rescue analgesia indicating that buprenorphine in combination with dental nerve blocks and NSAIDs may not provide adequate analgesia in some individuals. These findings were also reported after the administration of hydromorphone in cats undergoing dental extractions highlighting that severe oral disease and dental extractions produce severe pain postoperatively requiring frequent and long-lasting administration of opioids (article 1). In this study, an agonist of opioid receptors (hydromorphone) was administered as rescue analgesia in cats pretreated with a partial agonist of μ opioid receptors (buprenorphine). The combination of these two opioid analgesic drugs may be suboptimal and less than ideal. However, pain assessment was continuously performed to ensure patient comfort and to confirm that hydromorphone had been effective.

In this study, cats were included based on the number and location of tooth extraction as previously reported (article 1). In the aforementioned study, the severity of oral disease (minimal versus severe) was defined as dental scores ≤ or > 7, respectively, and 91.7% of the cats with severe oral disease required rescue analgesia even after the administration of hydromorphone in the premedication in combination with local anesthetic blocks and NSAIDs. In this study, the cut-off for dental scores was lower (i.e. ≥ 6) than in our previous study because the authors felt that this lower score already produces enough postoperative pain and inflammation allowing to study different analgesic treatments. A lower score also facilitated patient recruitment. However, this could explain the lower prevalence of rescue analgesia in this study (approximately 30%) versus the previous one

using hydromorphone in cats. The group allocation was performed randomly, and all demographic data and the dental parameters indicating the severity of oral disease were not different between treatment groups, which would make it reasonable to compare the analgesic efficacy of two treatments.

There are limitations in this study. Firstly, the pain evaluations were performed based on the time points after extubation time and not the administration of buprenorphine in the morning of day 1. Therefore, the patients were evaluated at different time points because of the different duration of surgery. However, anesthetic, procedure and surgical times were not significantly different between groups minimizing this potential bias in pain assessment. Secondly, the doses, concentrations, and routes of administration are different between Vetergesic and Simbadol which may influence their analgesic efficacy in cats. Simbadol is a high-concentration formulation of buprenorphine (1.8 mg/mL) approved for SC administration using high doses of the drug (0.24 mg/kg) whereas Vetergesic presentation has a lower concentration (0.3 mg/mL) and lower recommended doses of administration (0.02 mg/kg IM). It may be arguable that comparisons between the two drugs using such dosage regimens are not appropriate. According to previous studies, Simbadol (0.24 mg/kg SC) and standard concentrations of buprenorphine (0.3 mg/mL; 0.02 mg/kg IM) have different elimination half-life (12.3 hours and 7.7 hours), time to peak plasma concentrations (0.08 hour and 0.05 hour) and duration of antinociceptive effect (24 hours and between 1 and 4 hours when doses of 0.01-0.02 mg/kg are administered), respectively (139,162,259). Although the route of administration could have been standardized (i.e. subcutaneously), the SC administration of buprenorphine at 0.3 mg/mL did not produce a thermal antinociceptive effect when compared with IM or IV (139). Thirdly, resentment to drug administration was evaluated using a dichotomized means of assessment (i.e. presence or absence). To the authors' knowledge, there are no validated means of evaluating resentment to drug administration in cats. Resentment should ideally have been evaluated by an observer who was not aware of the treatment by using a validated scale, if one existed. The resentment to drug administration was likely higher in the Vetergesic group due to the number of injections using the IM route of administration as previously discussed. A more appropriate comparison would involve at least sham/placebo injections three times a day in the Simbadol group, however this was

not done to avoid unnecessary added stress to these cats. Finally, pain scores were excluded from statistical analysis after rescue analgesia which could decrease the power of the study and introduce selection bias. However, prevalence of rescue analgesia was used as an important outcome and it was not significantly different between groups corroborating our findings.

Conclusion

This study showed that both Simbadol and Vetergesic produced similar analgesic effects when using a multimodal analgesic protocol including local anesthetic nerve blocks and meloxicam in cats undergoing dental extractions. However, pain scores in the Vetergesic, but not in the Simbadol group, were still significantly higher in the morning of day 2 when compared with baseline values. This potentially indicates that Simbadol may present longer-sustained analgesic effects than Vetergesic with the dosage regimens used in this study. The frequency and route of drug administration with Vetergesic (i.e. every 8 hours IM) may induce more resentment (i.e. aversive behaviors) than Simbadol (i.e. every 24 hours SC).

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Supporting Information

S1 File. Raw data (https://doi.org/10.1371/journal.pone.0230079.s001)

Combined Discussion and Conclusion

A multidisciplinary study that involved pain and behavior assessment, nutrition, and inflammatory cytokines revealed specific clinical signs associated with dental pain in cats. Severe oral disease and multiple tooth extractions produce severe postoperative pain, and the long-term multimodal analgesic protocol including opioids, local anesthetics and NSAIDs are essential. The signs of food intake and oral pain-induced behaviors could be used at home by cat owners, and these signs should be the triggers to bring these patients to the hospital setting. For assessment of oral pain during hospitalization, the FGS could be an option in terms of the reliability and ease of use. The analgesic protocol that involves a high-concentrated formulation of buprenorphine (i.e. Simbadol, 1.8 mg/mL) produces a similar analgesic effect as the regular concentrated formulation (i.e. Vetergesic, 0.3 mg/mL) without resentment during the administration.

In the studies, the group allocation of oral disease was performed by using a dental scoring system that was developed based on the types and the number of extracted teeth. Since the scoring system is not validated, it is still not clear if the points given to each tooth extraction were appropriate. For example, in current studies, extraction of each incisor and 1st premolar tooth alone did not receive a score, and the point either 1 or 2 was given when the total number of extracted incisor and 1st premolar tooth was 1 to 6 or ≥ 7, respectively. It means that cats with one or six teeth extractions received the same scores in this case. Therefore, the degree of pain evoked after extraction of these teeth might be under/overestimated. Our first experiment (i.e. article 1) could differentiate the severity of oral disease between minimal and severe groups in terms of the prevalence of rescue analgesia. further study investigating the reliability and validity of the dental scoring system would be warranted.

In our studies, postoperative 4 hours was the time point most cats in the severe oral disease group required rescue analgesia at the day of surgery (5/12 cats and 3/24 cats in articles 1 and 4, respectively) even the cats had received multimodal analgesia protocol including opioid, dental nerve blocks and meloxicam. This time point would be the timing of offset duration of local anesthetics, and the results highlight that continuous

assessment and treatment of postoperative pain are essential, and attention should be paid if it is the day of surgery in cats undergoing multiple tooth extractions.

In articles 1 (multidisciplinary study) and 4 (buprenorphine study), pain assessment was always performed by using CMPS-F that was the only instrument validated at that time. As described in the discussion of article 2 (behavior study), however, some questions (i.e. questions 3 and 6) would not be applicable to dental pain because few cats had CMPS-F score ≥ 1 during the studies. Although the palpation of the painful area for question 6 was performed over the lips due to the cats' temperament and the methodology employed using an investigator who was blinded to disease severity, direct palpation of the wound area (gingiva) could help show the painful reaction. However, continuous assessment with the unusual manipulations (i.e. lifting the lips and palpation of gingiva) could produce a stress response, and it is not sure if reactions after the manipulations would be obtained from pain or from unpleasantness due to the palpation or manipulations themselves. The FGS could help to solve the problems because the manipulation is not necessary during the pain evaluation. The clinical applicability of real-time FGS evaluation was studied, and the study showed that there were only minimal bias and narrow limits of agreement between image-based and real-time assessments (248). Indeed, the real-time FGS evaluation was performed at the same time as the evaluation of CMPS-F in the buprenorphine study (260). In the study, the FGS did not detect a significant increase of pain at postoperative time points on the day of surgery in both Vetergesic and Simbadol groups, while CMPS-F scores were significantly higher at postoperative 4 and 8 hours on the day of surgery in both groups when compared with baseline. On the other hand, the FGS could detect pain in cats based on CMPS-F (i.e. CMPS-F score ≥ 5) at the same time points (unpublished data). Moreover, 3/7 cats that received rescue analgesia had reached the analgesic threshold of the FGS before reaching the threshold of CMPS-F, and 1 cat reached the threshold of FGS even CMPS-F score was < 5. Since these 4 cats had CMPS-F scores 3 and 4, these cats might be eligible to receive the rescue analgesia if CMPS-F could find detect painful signs from questions 3 and 6. Real-time assessment of FGS could be the gold-standard for assessment of dental pain in cats, and further study is warranted.

In the multidisciplinary study, the cats with severe oral disease had a significantly lower amount of postoperative soft food intake for 3 minutes and dry food intake for 3 minutes and 2 hours, and had significantly higher pain scores throughout the study when compared with those with minimal oral disease. The lower dry food intake would be due to the difficulty grasping the food, as shown in the behavior study. In a study of rats with experimentally induced dental injury, the bite force was significantly reduced to 61.9 ± 8.2, 51.9 ± 9.4 and 63.5 ± 11.5 % of baseline at 4, 24, and 48 hours post-pulp exposure, respectively, when compared with baseline, and the bite force was improved (95.2 ± 28.6 % of baseline) after the administration of morphine, which was not significantly different from the baseline (261). This result would support the finding of our studies that the difficulty grasping the dry food and decrease of dry food intake would be due to dental pain, and analgesic therapy is necessary postoperatively. Since the study was a clinical trial, the evaluations of pain and food intake could not be performed after discharge, and it is not clear if the differences between minimal and severe oral disease groups disappeared. A study in bears showed that animals undergoing dental treatment required 4 weeks to return to or superior to the baseline in terms of the duration of eating soft porridge and hard sugarcane (128). On the other hand, a rodent study found that food intake impairment associated with dental procedure was recovered only 8 days after the procedure (262). These results indicate that the time to return to the normal food intake varies depending on the patients, and careful monitoring after discharge would be required. Currently, the Composite Oral and Maxillofacial Pain Scale-Canine/Feline (COPS-C/F) that contains the evaluation by the owners was developed and validated (124). Since COPS-C/F includes the questions about food intake and oral pain behaviors, the scale would be useful for the post-discharge evaluation in cats undergoing multiple tooth extractions.

In the behavior study, a total of 36 hours of video filming was performed at 9 time points during the 7 days-hospitalization, and some oral pain-induced behaviors associated with general, playing, feeding and post-feeding behaviors could be identified. The video filming was usually performed in the daytime [1 pm and 6 pm on the day before the dental procedure (day 0), 6 am and 6 pm on the day of the dental procedure (day 1) and 8 am on days 2 to 6]. Because of the unbalanced filming time points, our study might have

overlooked some other pain-induced behaviors. For example, dental caries in humans affects the quality of sleep, and 53% of children with dental caries experienced sleep disturbance due to tooth pain at night (44). It is possible that quality of life and sleeping disturbances occur in cats with dental pain. An accelerometer-based motor activity which has been studied in cats with osteoarthritis-related chronic pain may be able to be applicable to cats with periodontal disease to evaluate their activity during the night (263).

In article 3 (FGS study), the analyses of inter-rater reliability and the effect of the caregiver's presence were performed by using the videos of days 1 and 6. Due to the exclusion of some images because of black cats and images obtained after rescue analgesia, the images of cats presenting moderate to severe pain were underrepresented, as discussed in article 3. If the videos from day 2 were also included, four videos of the cats scored CMPS-F ≥ 3 (i.e. moderate to severe pain) could be included, and which may have improved the results of the study. However, these were not included because the majority of cats were not painful, and we aimed an even number of images of painful and non-painful.

In article 4, hydromorphone was administered as the rescue analgesia even the cats had received buprenorphine that is a partial-μ agonist and has a high affinity to the receptor as perioperative analgesia, which could be a concern. A study showed that pretreatment with buprenorphine impaired the magnitude of thermal antinociception during a fentanyl infusion in cats (264). Similar findings were observed in a study in dogs where pretreatment with buprenorphine followed by sufentanil affected antinociception during surgery (265). On the other hand, IV administration of morphine following transdermal buprenorphine successfully relieved pain in 92.4% of people with cancer pain (266). Therefore, the results are conflicting. In the study, the most important thing was to confirm the outcome of rescue analgesia. Pain scoring was performed 30 minutes after rescue analgesia to ensure that hydromorphone had an appropriate analgesic effect. There is still a controversy whether buprenorphine should be administered instead of hydromorphone for feline analgesia of dental patients. If buprenorphine has failed to provide analgesia and might present a ceiling effect, especially in the case where large doses were administered (i.e. Simbadol), it is not clear whether it would be better to repeat

an opioid drug that could not provide appropriate analgesia (buprenorphine) than giving doses of a full μ-agonist of opioid receptors (hydromorphone) that could potentially displace buprenorphine from its receptors.

In the study of article 4, the prevalence of rescue analgesia in cats undergoing multiple tooth extractions was lower than in article 1 (30.4% vs. 91.7%) even when the number of extracted teeth and the dental scores were similar between the studies. This could indicate that long-term perioperative multiple administration of analgesics (Vetergesic 0.02 mg/kg every 8 hours or Simbadol 0.24 mg/kg every 24 hours) is important to decrease the prevalence of rescue analgesia as previously described.

In conclusion, the improvement of animal welfare has become a priority in veterinary medicine in recent years. During my Ph.D work, we have investigated pain-induced behaviors in cats undergoing dental extractions as well as postoperative pain scores and the need of supplemental analgesia. This work also reported inter-rater reliability of the FGS in these patients. Finally, the analgesic efficacy of two formulations of buprenorphine was compared using multidisciplinary approach.

Multiple tooth extractions cause severe pain and require aggressive perioperative pain management as shown in the studies. In feline medicine, however, a lot of cats undergoing multiple tooth extractions are discharged on the day of surgery with prescription of NSAIDs for a few days, and assessment of dental pain is not performed appropriately. The research has shown the importance of perioperative pain management and evidence of specific signs associated with oral disease in cats in the fields of nutrition and animal behavior and the utility of facial expression-based pain scale that are applicable by veterinarians but also potentially by owners. Postoperative pain management is essential in the aspect of feline welfare. Long-term administration of opioids (e.g. buprenorphine) and NSAIDs, especially up to 48 hours after dental treatment is necessary for pain management of the feline dental patient. In addition to the specific signs associated with dental pain reported herein, deepening the knowledge in this field is essential to further understanding of feline dental pain and its impact.

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