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Environmental risk factors for the development of oral squamous cell carcinoma in cats

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Abstract

Background: Risk factors for oral squamous cell carcinoma (OSCC) in cats are derived from a single study dated almost 20 years ago. The relationship between inflammation of oral tissues and OSCC is still unclear.

Objectives: To investigate previously proposed and novel potential risk factors for OSCC development, including oral inflammatory diseases.

Animals: Hundred cats with OSCC, 70 cats with chronic gingivostomatitis (CGS), 63 cats with periodontal disease (PD), and 500 controls.

Methods: Prospective, observational case-control study. Cats with OSCC were compared with an age-matched control sample of client-owned cats and cats with CGS or PD. Owners of cats completed an anonymous questionnaire including demographic, environmental and lifestyle information.

Results: On multivariable logistic regression, covariates significantly associated with an increased risk of OSCC were rural environment (OR: 1.77; 95% CI: 1.03-3.04; $P = .04$), outdoor access (OR: 1.68; 95% CI: 1.07-2.63; $P = .02$), environmental tobacco smoke (OR: 1.77; 95% CI: 1.05-3; $P = .03$), and petfood containing chemical additives (OR: 1.98; 95% CI: 1.04-3.76; $P = .04$). Risk factors shared with CGS and PD were outdoor access and petfood containing chemical additives, respectively. A history of oral inflammation was reported in 35% of cats with OSCC but did not emerge as a risk factor.

Conclusions and Clinical Importance: The study proposes novel potential risk factors for OSCC in cats. Although a history of inflammatory oral disease was not significantly more frequent compared with random age-matched controls, OSCC shared several risk factors with CGS and PD.

ABBREVIATIONS: CGS, chronic gingivostomatitis; CI, confidence interval; ETS, environmental tobacco smoke; FeLV, feline leukemia virus; FIV, feline immunodeficiency virus; OR, odds ratio; OSCC, oral squamous cell carcinoma; PD, periodontal disease.

KEYWORDS
canned food, diet, epidemiology, feline chronic gingivostomatitis, feline oral cancer, periodontal disease, squamous cell carcinoma, tobacco smoke

1 | INTRODUCTION

Squamous cell carcinoma is the most common oral tumor in cats, accounting for 70% to 80% of oral neoplasms. Despite multimodal treatment, the prognosis for this fast-growing, invasive tumor remains grave. This is mainly due to the fact that oral squamous cell carcinoma (OSCC) in cats is not detected until it has reached an advanced stage. Thus, early diagnosis is crucial for improving the survival rate.[1,2] Identification of environmental risk factors and screening surveillance of the subjects at risk might effectively contribute to tumor prevention and early detection, and this might translate in an increased opportunity for treatment.

It is likely that changes in management, feeding, housing, and treatment of cats have resulted in alternations to risk factors for OSSC since earlier studies.[3] The identified risk factors include environmental tobacco smoke (ETS) and use of flea collar, possibly because of oral carcinogen exposure during grooming.[3] Additionally, consumption of canned cat food and canned tuna has been linked to the development of this disease.[3] The proposed mechanisms for this association include differences in the nutrient content of these foods; alternatively, a high intake of canned cat food could result in poorer dental hygiene, favoring tartar build-up, production of bacterial toxins and oral inflammation, which might eventually promote neoplastic transformation.[3,4]

There is an association between oral inflammation and cancer in people and this was recently suggested in cats by the observation of altered DNA methylation profile of tumor-related genes in cats with stomatitis.[5-8] Inflammatory lesions of the oral cavity have a high prevalence in elderly cats. It has been estimated that between 50% and 90% of cats older than 4 years of age have some form of oral disease, with the most common being gingivitis, periodontal disease (PD) and tooth resorption.[9-12]

Chronic gingivostomatitis (CGS) is another oral inflammatory disease commonly encountered in cats, with a prevalence of 0.7-12%.[13] Lesions are characterized by a mixed proliferative and ulcerative phenotype and are typically located lateral to the palatoglossal folds.[9,13-15] The etiology of CGS is elusive and the pathogenesis is characterized by inappropriate immune response to an unknown antigenic stimulus, although underlying viral and bacterial etiologies have been proposed, including feline immunodeficiency virus (FIV), feline leukemia virus (FeLV), and feline calicivirus.[16,17]

In order to re-evaluate the previously proposed risk factors and to investigate further potential risk factors, including oral inflammation, we performed an observational epidemiological case-control study on cats with confirmed OSCC. Results were compared with an age-matched random sample of client-owned healthy cats and with 2 groups of cats having, respectively, CGS and PD.

2 | MATERIALS AND METHODS

2.1 | Retrospective study

The prospective epidemiological analysis was preceded by a retrospective analysis on all cases of OSCC diagnosed at the Pathology Service of the Department of Veterinary Medical Sciences, University of Bologna, from 2000 to date in order to contribute to the demographic characterization of the cats affected by this tumor with a larger sample size. Information was retrieved from specimen submission forms or medical records, and included breed, age, sex, FIV/FeLV status and history of oral inflammatory disease (dating back at least 1 year before diagnosis). Differences in the distribution of the above variables according to tumor location were investigated.

2.2 | Prospective study

Cats with a cytological or histological diagnosis of OSCC over a 3-year period (2018-2020) were prospectively included upon completion of an anonymous online questionnaire by the owner, including demographic information, lifestyle, diet, and living environment (Appendix S1). Owners were asked to refer to the entire life frame of the cat preceding the diagnosis of OSCC. If the cat had been adopted less than 3 years before SCC diagnosis, the case was excluded from the analysis.

Regarding coat color, a solid black coat was evaluated as a potential risk factor, since nicotine is known to have a high affinity for melanin, which is produced in the hair bulb and incorporated into the cortex of darker hair.[18-22] Cat food was classified as "market" (consisting in economical preparations with a selling price below € 4.00-5.00/kg, with no fixed ingredient formulation and sold by discount and mass-market retailers) or "premium" (consisting in more costly preparations, with a selling price higher than € 4.00-5.00/kg, with fixed ingredient formulation and sold in specialized stores). Cat food was also classified based on the content of artificial additives (coloring agents, flavor enhancers and preservatives) or not. In particular, brands that explicitly declared not to use chemical additives in their products were considered as low exposure, whereas all the others were considered high exposure. Parasite control agents were classified based on the spectrum of action of the active ingredients (endoparasiticide, ectoparasiticide, or both).

Enrolled cases had been referred to the Pathology Unit of the Department of Veterinary Medical Sciences (University of Bologna, Italy) from different veterinary facilities in Italy participating in a prospective study for the assessment of genetic and epigenetic alterations in OSCC ("Salice" project). Compilation of the questionnaire by cat owners was required for inclusion.

To create the control group, the same questionnaire was proposed to random cat owners via social networks. Cats without a previous or concurrent diagnosis of OSCC were eligible for inclusion. In the questionnaire, owners had to confirm that the cats had never suffered from oral tumors. After collecting the information, controls were filtered by age to match the OSCC sample. The choice of social networks for collecting control questionnaires was dictated by several needs:

- the need of recruiting cases from a wider geographical area;
- the need of recruiting cats outside the context of veterinary clinics, to avoid the selection of subgroups with specific diseases or owners more attentive to the health of their cats;
- the need of keeping the compilation of questionnaires as much anonymous and spontaneous as possible, and therefore potentially more reliable.

During the same time interval (2018-2020), the owners of cats referred to the odontostomatology units of the Department of Veterinary Medical Sciences (University of Bologna) and "I Portoni Rossi" and "Clinica Veterinaria Serenissima" private veterinary clinics for CGS and PD were also prospectively requested to fill out the same questionnaire.

Chronic gingivostomatitis was characterized by bilateral ulcerative or ulcero-proliferative mucosal lesions located caudally to the dental arch and lateral to the palatoglossal folds or in the oropharyngeal area, extending to the gingiva in a part of cases. PD was characterized by focal, multifocal, or generalized ulcerative lesions of the gingiva and adjacent oral mucosa, associated with plaque and dental calculus deposition.

2.3 | Statistical analysis

Summary statistics were presented as median (range) or as frequencies and percentages.

For the retrospective analysis, differences in signalment (ie, breed [purebred vs mixed], sex [male vs female], and age), FIV/FeLV status (positive vs negative), and history of oral inflammatory disease (yes vs no) according to tumor location (mandibular gingiva, maxillary gingiva, tongue, other locations) were investigated by means of χ^2 or Fisher's exact tests for categorical variables, or the Kruskal-Wallis test for continuous variables.

For the prospective analysis, the association of relevant demographic characteristics and lifestyle factors with diagnosis (OSCC, CGS, and PD) was assessed through binary logistic regression analysis; the effect size of covariates was expressed by odds ratios (ORs) with 95% confidence intervals (CIs), and the presence of systematic differences (ie, statistical significance) was assessed using the 2-sided Wald test. The variables considered in univariable regression analysis included breed (purebred, mixed), haircoat length (short, long), haircoat color (solid black, other), sex, reproductive status (intact, spayed/neutered), living environment (rural, other), outdoor access (yes, no), cohabitation with other cats (yes, no), indoor smoking owners (yes, no), type of diet (wet diet ≥50%, other), exposure to chemical additives in cat food (low, high), cat food price category (premium, market), diet including canned tuna (no, yes), diet including homemade food (no, yes), use of antiparasitic products (none/occasional, regular), antiparasitic action (endectocide, other), lack of vaccination (yes, no), history of oral inflammatory diseases (yes, no [evaluated only in OSCC and controls]), FIV and FeLV status (positive, negative).

Covariates with a *P*-value <.1 on univariate test were included in the multivariate (adjusted) regression model. There was no evidence of significant interactions (ie, moderating effects) between covariates, and no multi-collinearity issues were found.

All data were analyzed using Stata version 15 (StataCorp. 2001. Statistical Software: Release 15. College Station, TX: Stata Corporation). The significance level was set at 5%, and pairwise deletion of missing data was used.

3 | RESULTS

3.1 | Retrospective study

The total number of retrospectively collected OSCC cases was 594, including 526 mixed breed (90%) and 61 purebred cats (10%). In 7 cats, breed was not reported. In the purebred group, the most represented breeds were Persian (n = 29; 48%) Maine Coon (n = 12; 20%), Chartreux (n = 8; 13%), and Siamese (n = 5; 8%). Males were 266 (44%) and females were 328 (56%). The median age was 13 years (range, 1-21). Data regarding tumor anatomic location were available in 567 (95%) cases; in order of frequency, locations included tongue (n = 180; 32%), mandibular gingiva (n = 178; 31%), maxillary gingiva (n = 132; 23%), caudal oral mucosa (n = 30; 5%), vestibule (n = 27; 5%), and hard palate (n = 20; 4%). Information regarding FIV and FeLV status was available in 101 (17%) and 102 (17%) cases, respectively. Among them, 13 cats (13%) were FIV-positive and 3 (3%) were FeLV-positive. History of oral inflammation was retrieved in 143 (24%) cases, with 44 (31%) cats reporting recurrent gingivostomatitis, periodontitis, multiple dental extractions, or eosinophilic granuloma.

There were no significant differences in the distribution of data related to demographic information, FIV/FeLV status, and previous oral diseases based on tumor anatomic location. However, cats with mandibular OSCC had a percentage of oral diseases of 42% vs 32% of the remaining cats (Table 1).

3.2 | Prospective study

3.3 | OSCC

The owners of 100 cats with OSCC completed the questionnaire. There were 90 (90%) mixed-breed cats and 10 (10%) purebred cats, represented by 5 Chartreux, 2 Siamese, and 1 each of Persian, Maine

TABLE 1 Demographic information, FIV/FeLV status, and history of oral inflammation in 567 cats with oral squamous cell carcinoma stratified based on tumor location

Variable	Mandibular gingiva (n = 178)	Maxillary gingiva (n = 132)	Tongue (n = 180)	Other locations (n = 77)	*P*
Purebred[a]					.1
Yes	15 (9%)	17 (13%)	23 (13%)	3 (4%)	
No	160 (91%)	114 (87%)	154 (87%)	74 (96%)	
Sex					.54
Male	80 (45%)	65 (49%)	76 (42%)	31 (40%)	
Female	98 (55%)	67 (51%)	104 (58%)	46 (60%)	
Median age (range) [years]	13 (4-21)	13 (4-21)	12 (1-20)	13 (1-18)	.18
FIV status[a]					.86
Positive	4 (11%)	2 (9%)	4 (17%)	2 (14%)	
Negative	31 (89%)	21 (91%)	20 (83%)	12 (86%)	
FeLV status[a]					>.99
Positive	1 (3%)	0 (0%)	0 (0%)	0 (0%)	
Negative	34 (97%)	24 (100%)	24 (100%)	14 (100%)	
History of oral inflammation[a]					.12
Yes	22 (42%)	9 (24%)	7 (21%)	6 (33%)	
No	30 (58%)	29 (76%)	27 (79%)	12 (67%)	

[a]Percentage is based on the total number of cases with available information.

Coon, and Korat. Forty-six (46%) cats were neutered males and 54 (54%) were spayed females. The median age was 13 years (range, 5-21). The remaining data collected in the questionnaire are summarized in Table 2.

3.4 | CGS

Seventy cases of CGS were included in the study. There were 2 (3%) intact males, 34 (49%) neutered males, and 34 (49%) spayed females; 63 (90%) mixed-breed and 7 (10%) purebred cats, including Siamese (n = 2), Chartreux (n = 1), Himalayan (n = 1), Norwegian Forest Cat (n = 1), and Turkish Angora (n = 1). The median age was 9 years (range, 2-15). The other data collected in the questionnaire are summarized in Table 2.

3.5 | Periodontal disease

Sixty-three cats with PD were included in the study. There were 1 (2%) intact male, 36 (57%) neutered males, 2 (3%) intact females, and 24 (38%) spayed females; 53 (84%) mixed-breed and 10 (16%) purebred cats, including Persian (n = 3), Siamese (n = 2), Abyssinian (n = 2), Canadian Sphynx (n = 1), and Maine Coon (n = 1). The median age was 9 years (range, 2-18). The other data collected in the questionnaire are summarized in Table 2.

3.6 | Controls

The control sample included 500 cats, 7 (1%) intact males, 215 (43%) neutered males, 8 (1%) intact females, and 270 (54%) spayed females. There were 440 (88%) mixed-breed and 60 (12%) purebred cats; the most represented breeds included Persian (n = 19), Siamese (n = 13), Norwegian Forest Cat (n = 5), Maine Coon (n = 5), Chartreux (n = 3), and British shorthair (n = 3). The median age was 13 years (range, 7-21). The other data collected in the questionnaire are summarized in Table 2.

3.7 | Statistical analysis

On univariable logistic regression, compared with controls, covariates significantly associated with an increased risk of developing OSCC included rural living environment (OR = 2.02; 95% CI: 1.28-3.19; *P* = .003), outdoor access (OR = 1.86; 95% CI: 1.2-2.8; *P* = .006), wet diet ≥50% (OR = 1.81; 95% CI: 1.14-2.85; *P* = .01), consumption of cat food brands with high chemical additives (OR = 2.08; 95% CI: 1.19-3.64; *P* = .01), consumption of market (low-cost) cat food brands (OR 1.93; 95% CI: 1.24-3; *P* = .003), and positive FIV status (OR 2.44; 95% CI: 1-5.95; *P* = .05; Table 3).

Risk factors significantly associated with CGS were rural living environment (OR = 2.6; 95% CI: 1.55-4.38; *P* < .001), outdoor access (OR = 3.34; 95% CI 1.93-5.78; *P* < .001), cohabitation with other cats

TABLE 2 Data collected via an online anonymous questionnaire in a prospective epidemiological study on cats with oral squamous cell carcinoma and chronic oral inflammatory disease

Variable	OSCC (n = 100)	CGS (n = 70)	PD (n = 63)	Controls (n = 500)
Purebred				
Yes	10 (10%)	7 (10%)	10 (16%)	60 (12%)
No	90 (90%)	63 (90%)	53 (84%)	440 (88%)
Haircoat				
Short	87 (87%)	61 (87%)	52 (83%)	400 (80%)
Long	13 (13%)	9 (13%)	11 (17%)	100 (20%)
Haircoat color[a]				
Solid black	17 (17%)	8 (12%)	11 (17%)	59 (12%)
Other	83 (83%)	61 (88%)	52 (83%)	441 (88%)
Sex				
Male	46 (46%)	36 (51%)	37 (59%)	222 (44%)
Female	54 (54%)	34 (49%)	26 (41%)	278 (56%)
Reproductive status				
Intact	0 (0%)	2(3%)	3 (5%)	15 (3%)
Spayed/neutered	100 (100%)	68 (97%)	60 (95%)	485 (97%)
Median age (range) [years]	13 (5-21)	9 (2-15)	9 (2-18)	13 (7-21)
Living environment[a]				
Urban	25 (25%)	12 (17%)	21 (33%)	177 (35%)
Small town/suburb	37 (37%)	27 (39%)	24 (38%)	209 (42%)
Rural	37 (37%)	30 (44%)	18 (29%)	114 (23%)
Outdoor access				
No	42 (42%)	20 (29%)	38 (60%)	286 (57%)
Yes	50 (50%)	44 (63%)	25 (40%)	200 (40%)
Outdoor only	8 (8%)	6 (8%)	0 (0%)	14 (3%)
Cohabitation with other cats[a]				
No	34 (35%)	8 (12%)	18 (29%)	207 (41%)
Yes	63 (65%)	60 (88%)	45 (71%)	293 (59%)
Number of cigarettes smoked per day in house				
0	69 (69%)	56 (79%)	49 (78%)	389 (79%)
1 to 10	18 (18%)	9 (13%)	9 (14%)	63 (13%)
>10	13 (13%)	5 (8%)	5 (8%)	48 (8%)
Diet[a]				
Dry prevalent	32 (33%)	30 (43%)	20 (32%)	227 (45%)
Other	66 (67%)	39 (57%)	42 (68%)	273 (55%)
Chemical additives in cat food[a]				
Low	17 (18%)	17 (25%)	12 (20%)	151 (31%)
High	80 (82%)	50 (75%)	48 (80%)	341 (69%)
Cat food price category[a]				
Premium	44 (45%)	42 (63%)	42 (70%)	303 (62%)
Market	53 (55%)	25 (37%)	18 (30%)	189 (38%)
Canned tuna[a]				
No	36 (36%)	31 (44%)	28 (44%)	226 (45%)
Yes	63 (64%)	39 (56%)	35 (56%)	274 (55%)
Homemade food				
No	80 (80%)	59 (84%)	54 (86%)	419 (84%)
Yes	20 (20%)	11 (16%)	9 (14%)	81 (16%)

TABLE 2 (Continued)

Variable	OSCC (n = 100)	CGS (n = 70)	PD (n = 63)	Controls (n = 500)
Parasite control				
None/occasional	36 (36%)	13 (19%)	25 (40%)	200 (40%)
Regular	64 (64%)	57 (81%)	38 (60%)	300 (60%)
Antiparasitic drug formulation[b]				
Spot-on drops	59 (59%)	52 (74%)	35 (56%)	276 (55%)
Collar	3 (3%)	1 (1%)	2 (3%)	27 (5%)
Oral products	4 (4%)	5 (7%)	1 (2%)	9 (2%)
Antiparasitic action[c]				
Ectocide	44 (76%)	29 (53%)	20 (53%)	199 (72%)
Endectocide	14 (24%)	26 (47%)	18 (47%)	78 (28%)
Trivalent vaccine[a]				
Never given	12 (12%)	24 (35%)	16 (25%)	82 (16%)
Occasional/limited to the first years of life	52 (52%)	24 (35%)	26 (41%)	239 (48%)
Regular	36 (36%)	21 (30%)	21 (33%)	179 (36%)
History of oral inflammation[a]		-	-	
No	64 (65%)			352 (70%)
Yes	34 (35%)			148 (30%)
FIV status[a]				
Negative	84 (91%)	46 (73%)	50 (94%)	385 (96%)
Positive	8 (9%)	17 (27%)	3 (6%)	15 (4%)
FeLV status[a]				
Negative	92 (100%)	59 (94%)	54 (100%)	395 (99%)
Positive	0 (0%)	4 (6%)	0 (0%)	5 (1%)

Abbreviations: CGS, chronic gingivostomatitis; OSCC, oral squamous cell carcinoma; PD, periodontal disease.
[a]Percentage is based on the total number of cases with available information.
[b]More than 1 formulation per cat admitted. Percentage calculated on the total number of cats.
[c]Percentage calculated on the total number of cats undergoing regular parasite control with known antiparasitic drugs.

(OR = 5.3; 95% CI: 2.48-11.32; $P < .001$), regular use of antiparasitic products (OR = 2.92; 95% CI: 1.56-5.48; $P = .001$), use of spot-on formulations (OR = 2.48; 95% CI: 1.4-4.41; $P = .002$), use of oral antiparasitic products (OR = 4.26; 95% CI: 1.38-13.11; $P = .01$), use of endectocides vs ectocides (OR = 2.29; 95% CI: 1.27-4.13; $P = .006$), lack of vaccination (OR = 2.72; 95% CI: 1.57-4.71; $P < .001$), positive FIV status (OR = 9.49; 95% CI: 4.44-20.26; $P < .001$), and positive FeLV status (OR = 5.36; 95% CI: 1.4-20.52; $P = .01$; Table 3).

Risk factors significantly associated with PD included male sex (OR = 1.78; 95% CI: 1.05-3; $P = .03$), wet diet ≥50% (OR = 1.8; 95% CI: 1.02-3.13; $P = .04$), and use of endectocides vs ectocides (OR = 2.05; 95% CI: 1.12-3.72; $P = .02$; Table 3).

There was no significant difference between OSCC and CGS regarding rural living environment ($P = .43$) and outdoor access ($P = .19$), nor between OSCC and PD regarding wet diet ≥50% ($P < .99$).

On multivariable analysis, covariates retaining statistical significance were rural living environment, outdoor access, indoor smoking, and consumption of commercial cat food brands with high chemical additives for OSCC; covariates associated with CGS included outdoor access, cohabitation with other cats, endectocide antiparasitic drugs, and positive FIV status, whereas male sex, cohabitation with other cats, wet diet ≥50%, and consumption of cat food brands with high chemical additives were associated with PD (Tables 4-6).

4 | DISCUSSION

Although there is abundant literature on OSCC in cats, none of the published studies included samples of adequate size for a meaningful demographic characterization of the population affected by this tumor. Thus, this prospective epidemiological study was preceded by a retrospective analysis on almost 600 cases, collected over a 20-year period, to investigate potential differences in demographic information, FIV/FeLV status, and history of oral inflammation according to tumor location. Notably, 30% of cats were reported with a previous history of oral inflammatory disease, with a higher percentage (42%) observed in cats with mandibular tumors; however, no statistically significant differences were observed in the investigated variables according to location.

TABLE 3 Univariable logistic regression for the evaluation of risk factors associated to the development of oral squamous cell carcinoma and chronic oral inflammatory lesions in cats

	OSCC		CGS		PD	
Variable	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
Purebred	0.82 (0.40-1.65)	.57	0.82 (0.36-1.86)	.63	1.38 (0.67-2.86)	.38
Long haircoat	0.6 (0.32-1.11)	.11	0.59 (0.28-1.23)	.16	0.85 (0.43-1.68)	.63
Solid black haircoat	1.53 (0.85-2.76)	.16	0.98 (0.45-2.15)	.96	1.58 (0.78-3.2)	.2
Male sex	1.07 (0.69-1.64)	.77	1.33 (0.8-2.19)	.27	1.78 (1.05-3)	.03[a]
Neutering	-	-	1.05 (0.23-4.7)	.95	1.62 (0.45-5.75)	.46
Rural living environment	2.02 (1.28-3.19)	.003[a]	2.6 (1.55-4.38)	<.001[a]	1.35 (0.75-2.43)	.31
Outdoor access	1.86 (1.2-2.8)	.006[a]	3.34 (1.93-5.78)	<.001[a]	0.88 (0.52-1.5)	.64
Cohabitation with other cats	1.31 (0.83-2.06)	.24	5.3 (2.48-11.32)	<.001[a]	1.77 (0.99-3.14)	.05
Indoor smoking	1.57 (0.98-2.53)	.06	0.88 (0.47-1.63)	.68	1 (0.53-1.88)	.99
Wet diet ≥50%	1.81 (1.14-2.85)	.01[a]	1.1 (0.7-1.84)	.7	1.78 (1.02-3.13)	.04[a]
Chemical additives in cat food	2.08 (1.19-3.64)	.01[a]	1.3 (0.73-2.33)	.37	1.77 (0.92-3.43)	.09
Low-cost cat food	1.93 (1.24-3)	.003[a]	0.9 (0.53-1.52)	.68	0.69 (0.38-1.23)	.21
Canned tuna in diet	1.44 (0.92-2.25)	.11	1.04 (0.63-1.72)	.89	1.03 (0.61-1.75)	.91
Homemade food in diet	1.29 (0.75-2.23)	.36	0.96 (0.49-1.92)	.92	0.86 (0.41-1.82)	.7
Regular parasite control	1.18 (0.76-1.85)	.46	2.92 (1.56-5.48)	.001[a]	1.01 (0.59-1.73)	.96
Endectocide antiparasitic drugs	0.85 (0.45-1.61)	.62	2.29 (1.27-4.13)	.006[a]	2.05 (1.12-3.72)	.02[a]
Lack of vaccination	0.7 (0.36-1.33)	.27	2.72 (1.57-4.71)	<.001[a]	1.74 (0.94-3.21)	.08
History of oral inflammation	1.26 (0.8-2)	.32	-	-	-	-
Positive FIV status	2.44 (1-5.95)	.05[a]	9.49 (4.44-20.26)	<.001[a]	1.54 (0.43-5.51)	.51
Positive FeLV status	-	-	5.36 (1.4-20.52)	.01[a]	-	-

Note: Analyses were relative to 500 control cats.
Abbreviations: CGS, chronic gingivostomatitis; OSCC, oral squamous cell carcinoma; PD, periodontal disease.
[a]Significant.

TABLE 4 Multivariable logistic regression for the evaluation of risk factors associated to the development of oral squamous cell carcinoma in cats

Variable	**OR (95% CI)**	***P***
Rural living environment	1.77 (1.03-3.04)	.04[a]
Outdoor access	1.68 (1.07-2.63)	.02[a]
Indoor smoking	1.77 (1.05-3)	.03[a]
Wet diet ≥50%	1.58 (0.96-2.6)	.07
Chemical additives in cat food	1.98 (1.04-3.76)	.04[a]
Low-cost cat food	1.36 (0.81-2.31)	.24
Positive FIV status	2 (0.75-5.32)	.16

Note: Analyses were relative to 500 control cats.
[a]Significant.

TABLE 5 Multivariable logistic regression for the evaluation of risk factors associated to the development of caudal gingivostomatitis in cats

Variable	**OR (95% CI)**	***P***
Rural living environment	1.51 (0.98-2.34)	.06
Outdoor access	2.3 (1.26-4.25)	.01[a]
Cohabitation with other cats	4.86 (2.04-11.59)	<.001[a]
Regular parasite control	0.65 (0.27-1.53)	.32
Endectocide antiparasitic drugs	2.61 (1.28-5.29)	.01[a]
Lack of vaccination	1.73 (0.87-3.44)	.12
Positive FIV status	6.05 (2.39-15.3)	<.001[a]
Positive FeLV status	3.03 (0.54-16.86)	.21

Note: Analyses were relative to 500 control cats.
[a]Significant.

The prospective epidemiological study paralleled a research project for the development of a molecular diagnostic test for OSCC in cats. The main goal was to provide an update on environmental risk factors for this tumor in comparison with the previously published paper, dating back almost 2 decades.[3] The identification of potential risky behaviors might eventually contribute to the prevention of a disease that is unfortunately still incurable. A second aim was to investigate the possible relationship between oral inflammatory diseases and OSCC development, considering the increasing evidence of a link between chronic oral inflammation and OSCC in humans.[23,24] The biological link resides in the expression of genes that are implicated in both modulation and secretion of inflammatory mediators, and in survival and proliferation of cancer cells. Moreover, partially

TABLE 6 Multivariable logistic regression for the evaluation of risk factors associated to the development of periodontal disease in cats

Variable	OR (95% CI)	*P*
Male sex	1.8 (1.03-3.15)	.04[a]
Cohabitation with other cats	1.9 (1.03-3.52)	.04[a]
Wet diet ≥50%	1.8 (1-3.24)	.05[a]
Chemical additives in cat food	1.98 (1.01-3.91)	.05[a]
Endectocide antiparasitic drugs	1.41 (0.97-2.04)	.07
Lack of vaccination	1.66 (0.86-3.21)	.13

Note: Analyses were relative to 500 control cats.
[a]Significant.

similar epigenetic alterations have been identified between OSCC and stomatitis in cats.[8] Thus, the shared risk factors between OSCC and 2 groups of cats with chronic oral inflammatory disease were further evaluated.

Compared with earlier studies, we examined a larger sample in terms of both number of cases and controls. Furthermore, the control cases had been previously enrolled among subjects with chronic renal failure, leading to possible bias because of the restricted number of cases and the link between chronic kidney disease and uremia-associated stomatitis.[3,25] To minimize bias, in the present study we elected to enroll a random, age-matched control sample, recruited outside of veterinary healthcare facilities.

Regarding oral inflammatory diseases, we included 2 homogeneous groups of cats with CGS and PD, representing the most frequent non-neoplastic feline oral diseases in the clinical setting. The etiology of CGS is still uncertain; the most likely hypothesis is that it can derive from an inappropriate immune response to oral antigenic stimulation. Possible triggers include food allergies and infections by several viral (eg, feline calicivirus, FeLV, FIV, feline herpesvirus) and bacterial (eg, Bartonella, plaque-associated Gram-negative bacteria) agents.[13-15,17,26-28] Among the reported predisposing factors, there are cohabitation with other cats, outdoor access, and straying.[9,10,29]

The etiology of PD is even less characterized, but it also appears to be linked to immune imbalances, with alteration of the oral microbiome and proliferation of Gram-positive anaerobic bacteria in dental calculi. FIV and FeLV infections can also predispose to the onset of PD, because of viral-induced immune suppression.[30-32] PD is one of the most common clinical finding in cats, this may be partly due to an increasing awareness of pet owners.[33] However, the greater consumption of soft food has been considered the main factor responsible for the decline of dental health in cats.[34-36] Conversely, the consumption of dry food is protective for plaque control by virtue of its physical properties (consistency, abrasiveness, and chewability).[33,36]

The present study confirmed most of the proposed risk factors for CGS, given that subjects within this group were more often FIV- or FeLV-positive, not routinely vaccinated, with outdoor access, living in a rural environment and cohabiting with other cats. For this category, a more frequent use of antiparasitic products was further observed, both as spot-on and oral formulations and with an endectocide spectrum, most likely related to the outdoor lifestyle of these animals. Furthermore, the reduced use of vaccines might have aided the transmission of agents involved in the pathogenesis of this disease.

Again, in agreement with the literature, among the risk factors identified for PD there was a low consumption of dry food. However, since the main treatment for this disease is tooth extraction, it remains to be established whether this dietary choice really represents the cause of this condition, or it is rather a consequence. Furthermore, multivariable regression analysis identified a higher consumption of commercial cat foods containing chemical additives as an additional independent risk factor.

Regarding OSCC, the survey highlighted multiple risk factors. Among those previously reported, we were able to confirm exposure to ETS. However, it must be emphasized that only 31% of subjects with OSCC had smoking owners, so for two-thirds of cases it is assumed that other factors were involved in tumorigenesis. In addition, it should be remembered that subjects could have been exposed to combustion products other than those of tobacco and not explicitly investigated in the questionnaire (eg, open field combustion of biomass, indoor wood burning, air-borne particulate matter). An additional risk factor that was confirmed in the present study is the consumption of canned cat food or, in our case, a diet consisting of half or more of wet industrial cat food. Analytic studies are warranted to investigate the carcinogenetic role of contaminants (eg, heavy metals, mycotoxins) or additives (eg, cassia gum, sodium nitrite, and chemical antioxidant such as butylated hydroxytoluene and hydroxyanisole) in cat food.[37] In the impossibility of performing such analysis, we relied on 2 further distinct indicators: price range, which was divided into 2 macro-categories ("market" and "premium") and the declared absence of chemical compounds by the producer. It should be noted that, according to the Code of Good Labelling Practice for Pet Food, produced by the European Pet Food Industry Federation, there is no obligation to declare additives with no legal maximum limit.[38] Additives of the functional groups "preservatives," "antioxidants," "flavorings," and "colorants" need not be declared by name but can be declared by only the respective functional group. This applies even when the level of the additive exceeds the recommended maximum level.[37] Therefore, we considered as low exposure only brands that explicitly declared not to use chemical additives in their products. A further limit for this classification is given by the fact that cats' diet is often variable and commonly includes different brands, so the data are based on an estimation of the most consumed commercial food brands during life. Nevertheless, relevant results emerged from these classifications, since both the consumption of pet food with high chemical additives and a market price range turned out to be significant risk factors in univariable analysis. Consumption of commercial food with chemical additives was further confirmed in multivariable survival analysis and was shared with the group of cats with PD.

A slight tendency to a greater development of OSCC in subjects using flea collars has been reported[3]; in the present study, it was not

possible to identify any significant association related to the type, formulation, or frequency of administration of antiparasitic products. However, it is likely that antiparasitic compounds have changed over years and it is possible that those used in the past were more toxic and are no longer on the market. Additionally, the use of antiparasitic collars is not frequent for cats in our country, and spot-on drops are the most widespread formulation in this species.

A high affinity of nicotine for melanin, which is incorporated into the cortex of darker hair, has been assumed in people.[18-21] For this reason, the possible association between black haircot and increased risck of OSCC was investigated. However, although the percentage of dark-coated cats was higher in OSCC, no significant increase in tumor risk was observed in the present study. Fifty percent of the purebred cats in the OSCC group were represented by Chartreux, vs 5% of the control group; this higher prevalence could depend either on the blue color (a dilution of black) of the coat of these subjects or on a genetic breed predisposition.

Another group of risk factors investigated in our study is inherent to the living environment. According to our results, cats with OSCC lived most frequently in rural areas and had outdoor access; both risk factors were shared with the CGS group. A further risk factor in common with CGS was a positive FIV status. An association between FIV and OSCC has been hypothesized.[39,40] It remains to be determined whether outdoor access in a rural environment could expose cats to higher levels of potentially carcinogenetic contaminants or whether this should be interpreted as a connection between CGS and OSCC.

In several cats with OSCC, previous episodes of oral diseases were reported, including severe CGS, caudal stomatitis, multiple dental extractions, oral ulcers and, in a few cases, oral eosinophilic granuloma. This pointed us toward a possible relationship between oral inflammation and carcinogenesis. However, based on the results of the survey, it was not possible to confirm this hypothesis, since the prevalence of previous oral problems in OSCC (35%) was only slightly higher than that of the control population (30%). Though it must be remembered that the questionnaire generically enquired the presence or absence of dental problems or oral inflammation. This did not allow to differentiate between the accumulation of tartar, a common problem in elderly cats such as those of the control population, and more severe lesions affecting the oral mucosa.

The results of this study suffer from the limitations inherent in observational epidemiological studies.

In addition, studies based on questionnaires might raise concerns regarding data consistency because of lack of interest of the respondents, level of education, time requirements, or sense of guilt. Anonymity should decrease the latter possibility and provide more reliable results. Finally, since the questionnaire was addressed to a wide audience of owners, exceedingly specific questions were avoided, which may have hampered a deeper understanding of the relationship between OSCC and oral diseases.

Papillomavirus infection was hypothesized to be another risk factor for OSCC in cats, considering its confirmed carcinogenic effect for human squamous cell carcinoma, and its presumptive causative role in a subset of cutaneous squamous cell carcinomas. However, results from different research groups were controversial, and its role in OSCC development has not yet been ultimately clarified.[41-43] Another factor that might be worthy of consideration is the oral microbiota, which has been associated with both feline and human oral inflammatory diseases and human oral cancer.[44,45] To the authors' knowledge, no information about feline oral microbiota in OSCC is currently available.

In conclusion, OSCC is likely a multifactorial disease, with several different risk factors contributing to its development. Our results were only partially consistent with those previously reported and included lifestyle risk factors (rural living environment, outdoor access), exposure to tobacco smoke and a predominantly wet diet, based on low-cost commercial food and containing chemical additives. Although the prevalence of previous inflammatory oral disease was not significantly higher in OSCC compared with a random age-matched control group, OSCC shared several risk factors with both CGS and PD. Further investigations on larger cohorts, along with molecular epidemiological studies, are warranted to ultimately exclude the existence of a biological link between these diseases.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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REFERENCES

1. Bilgic O, Duda L, Sánchez MD, Lewis JR. Feline oral squamous cell carcinoma: Clinical manifestations and literature review. *J Vet Dent*. 2015;32:30-40.

2. Liptak JM. Cancer of gastrointestinal tract. In: Vail DM, Thamm DH, Liptak JM, eds. *Withrow and MacEwen's small animal clinical oncology*. 6th ed. St. Louis, MO: Elsevier Saunders; 2020:432-491.
3. Bertone ER, Snyder LA, Moore AS. Environmental and lifestyle risk factors for oral squamous cell carcinoma in domestic cats. *J Vet Intern Med*. 2003;17:557-562.
4. Studer E, Stapley RB. The role of dry food in maintaining healthy teeth and gums in the cat. *Vet Med Small Anim Clin*. 1973;68:1124-1126.
5. Franco EL, Kowalski LP, Oliveira BV, et al. Risk factors 97 for oral cancer in Brazil: a case-control study. *Int J Cancer*. 1989;43:992-1000.
6. Moreno-López LA, Esparza-Gómez GC, González-Navarro A, Cerero-Lapiedra R, González-Hernández MJ, Domínguez-Rojas V. Risk of oral cancer associated with tobacco smoking, alcohol consumption and oral hygiene: a case-control study in Madrid, Spain. *Oral Oncol*. 2000;36:170-174.
7. Garrote LF, Herrero R, Reyes RM, et al. Risk factors for cancer of the oral cavity and oro-pharynx in Cuba. *Br J Cancer*. 2001;85:46-54.
8. Renzi A, Morandi L, Lenzi J, et al. Analysis of DNA methylation and TP53 mutational status for differentiating feline oral squamous cell carcinoma from non- neoplastic mucosa: a preliminary study. *Vet Comp Oncol*. 2020;18:825-837.
9. Healey KAE, Dawson S, Burrow R, et al. Prevalence of feline chronic gingivo-stomatitis in first opinion veterinary practice. *J Feline Med Surg*. 2007;9:373-381.
10. Girard N, Servet E, Biourge V, Hennet P. Periodontal health status in a colony of 109 cats. *J Vet Dent*. 2009;26:147-155.
11. O'Neill DG, Church DB, McGreevy PD, Thomson PC, Brodbelt DC. Prevalence of disorders recorded in cats attending primary-care veterinary practices in England. *Vet J*. 2014;202:286-291.
12. Falcão F, Faísca P, Viegas I, de Oliveira JT, Requicha JF. Feline oral cavity lesions diagnosed by histopathology: a 6-year retrospective study in Portugal. *J Feline Med Surg*. 2020;22:977-983.
13. Lee DB, Verstraete FJM, Arzi B. An update on feline chronic gingivostomatitis. *Vet Clin North Am Small Anim Pract*. 2020;50:973-982.
14. Lommer MJ, Verstraete FJM. Concurrent oral shedding of feline calicivirus and feline herpesvirus 1 in cats with chronic gingivostomatitis. *Oral Microbiol Immunol*. 2003;18:131-134.
15. Winer JN, Arzi B, Verstraete FJM. Therapeutic management of feline chronic gingivostomatitis: a systematic review of the literature. *Front Vet Sci*. 2016;3:54.
16. Belgard S, Truyen U, Thibault JC, Sauter-Louis C, Hartmann K. Relevance of feline calicivirus, feline immunodeficiency virus, feline leukemia virus, feline herpesvirus and *Bartonella henselae* in cats with chronic gingivostomatitis. *Berl Munch Tierarztl Wochenschr*. 2010;123:369-376.
17. Vapniarsky N, Simpson DL, Arzi B, et al. Histological, immunological, and genetic analysis of feline chronic gingivostomatitis. *Front Vet Sci*. 2020;7:310.
18. Mizuno A, Uematsu T, Oshima NM, Nakashima M. Analysis of nicotine content of hair for assessing individual cigarette-smoking behaviour. *Ther Drug Monit*. 1993;15:99-104.
19. Gerstenberg B, Schepers G, Voncken P, Völkel H. Nicotine and cotinine accumulation in pigmented and unpigmented rat hair. *Drug Metab Dispos*. 1995;23:143-148.
20. Uematsu T, Mizuno A, Nagashima S, Oshima A, Nakamura M. The axial distribution of nicotine content along hair shaft as an indicator of changes in smoking behaviour: evaluation in a smoking-cessation programme with or without the aid of nicotine chewing gum. *Br J Clin Pharmacol*. 1995;39:665-669.
21. Al-Delaimy WK, Crane J, Woodward A. Is the hair nicotine level a more accurate biomarker of environmental tobacco smoke exposure than urine cotinine? *J Epidemiol Community Health*. 2002;56:66-71.
22. Apelberg BJ, Hepp LM, Avila-Tang E, et al. Racial differences in hair nicotine concentrations among smokers. *Nicotine Tob Res*. 2012;14:933-941.
23. Feller L, Altini M, Lemmer J. Inflammation in the context of oral cancer. *Oral Oncol*. 2013;49:887-892.
24. Gopinath D, Kunnath Menon RK, Veettil S, George Botelho M, Johnson NW. Periodontal diseases as putative risk factors for head and neck cancer: systematic review and meta-analysis. *Cancers*. 2020;12:1893.
25. Murphy BG, Bell CM, Soukup JW. Inflammatory lesions of the oral mucosa and jaws. *Veterinary oral and maxillofacial pathology*. 1st ed. Hoboken, NJ: Wiley-Blackwell; 2020:68-69.
26. Quimby JM, Elston T, Hawley J, Brewer M, Miller A, Lappin MR. Evaluation of the association of Bartonella species, feline herpesvirus 1, feline calicivirus, feline leukemia virus and feline immunodeficiency virus with chronic feline gingivostomatitis. *J Feline Med Surg*. 2008;10:66-72.
27. Dolieslager SM, Riggio MP, Lennon A, et al. Identification of bacteria associated with feline chronic gingivostomatitis using culture-dependent and culture-independent methods. *Vet Microbiol*. 2011;148:93-98.
28. Thomas R, Smith KC, Ostrander EA, Galibert F, Breen M. Chromosome aberrations in canine multicentric lymphomas detected with comparative genomic hybridisation and a panel of single locus probes. *Br J Cancer*. 2003;89:1530-1537.
29. Peralta S, Carney PC. Feline chronic gingivostomatitis is more prevalent in shared households and its risk correlates with the number of cohabiting cats. *J Feline Med Surg*. 2019;21:1165-1171.
30. Kornya MR, Little SE, Scherk MA, Sears WC, Bienzle D. Association between oral health status and retrovirus test results in cats. *J Am Vet Med Assoc*. 2014;245:916-922.
31. Harris S, Croft J, O'Flynn C, et al. A pyrosequencing investigation of differences in the feline subgingival microbiota in health, gingivitis and mild periodontitis. *PLoS ONE*. 2015;10(11):e0136986.
32. Thengchaisri N, Steiner JM, Suchodolski JS, Sattasathuchana P. Association of gingivitis with dental calculus thickness or dental calculus coverage and subgingival bacteria in feline leukemia virus- and feline immunodeficiency virus-negative cats. *Can J Vet Res*. 2017;81:46-52.
33. Perry R, Tutt C. Periodontal disease in cats: Back to basics – with an eye on the future. *J Feline Med Surg*. 2015;17:45-65.
34. Watson AD. Diet and periodontal disease in dogs and cats. *Aust Vet J*. 1994;71:313-318.
35. Gawor JP, Reiter AM, Jodkowska K, Kurski G, Wojtacki MP, Kurek A. Influence of diet on oral health in cats and dogs. *J Nutr*. 2006;136(7 Suppl):2021S-2023S.
36. Mata F. The choice of diet affects the oral health of the domestic cat. *Animals*. 2015;5:101-109.
37. Craig JM. Additives in pet food: are they safe? *J Small Anim Pract*. 2021;62:624-635.
38. FEDIAF. Code of Good labelling practice for pet food. 2018. http://www.fediaf.org/images/FEDIAF_Labelling_Code_October_2018_online_final.pdf#page=20
39. Hutson CA, Rideout BA, Pedersen NC. Neoplasia associated with feline immunodeficiency virus infection in cats of southern California. *J Am Vet Med Assoc*. 1991;199:1357-1362.
40. Magden E, Quackenbush SL, VandeWoude S. FIV associated neoplasms – a mini-review. *Vet Immunol Immunopathol*. 2011;143:227-234.
41. Munday JS, Sharp CR, Beatty JA. Novel viruses: update on the significance of papillomavirus infections in cats. *J Feline Med Surg*. 2019;21:409-418.
42. Altamura G, Cardeti G, Cersini A, et al. Detection of Felis catus papillomavirus type-2 DNA and viral gene expression suggest active

infection in feline oral squamous cell carcinoma. *Vet Comp Oncol*. 2020;18:494-501.
43. Chu S, Wylie TN, Wylie KM, et al. A virome sequencing approach to feline oral squamous cell carcinoma to evaluate viral causative factors. *Vet Microbiol*. 2020;240:108491.
44. Lamont RJ, Koo H, Hajishengallis G. The oral microbiota: dynamic communities and host interactions. *Nat Rev Microbiol*. 2018;16:745-759.
45. Rodrigues MX, Fiani N, Bicalho RC, Peralta S. Preliminary functional analysis of the subgingival microbiota of cats with periodontitis and feline chronic gingivostomatitis. *Sci Rep*. 2021;11:6896.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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