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Association of gastric lymphofollicular hyperplasia with *Helicobacter*-like organisms in dogs

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Abstract

Background: The relationships among gastric lymphoid follicular hyperplasia (GLFH), *Helicobacter*-like organisms (HLOs), and clinical signs have not been established in dogs.

Objectives: To evaluate the epidemiologic, clinical, endoscopic, and histopathologic findings associated with GLFH in dogs, and determine the association of GLFH with HLOs and the French Bulldog (FB) breed.

Animals: Two hundred eighty-eight dogs that underwent gastroscopy between 2013 and 2016.

Methods: Retrospective, cross-sectional study. Gastric biopsy samples were reviewed and scored for inflammation and HLOs. Dogs were divided into 3 groups: group 1 (63 FBs), group 2 (45 non-FB brachycephalic dogs), and group 3 (180 nonbrachycephalic dogs). Variables were evaluated for their association with GLFH.

Results: Univariate analysis determined that intact males, young age, vomiting, gastroscopic findings (discoloration, hemorrhage, and ulcers), and histopathologic findings (gastric lamina propria lymphocytic infiltration and HLO score) were associated with GLFH ($P \leq .03$). In the multivariate analysis, GLFH was associated with the HLO score (odds ratio [OR] > 5 for HLO scores 1-2 and >15 for HLO score of 3; $P < .001$), with vomiting (OR > 4; $P = .01$) but not with FB breed ($P = .76$) and age ($P = .1$). The HLO score was associated with younger age ($P < .001$).

Conclusion and Clinical Importance: The HLO score was associated with a high GLFH score. Vomiting was associated with GLFH. *Helicobacter*-like organisms are highly prevalent in young dogs and GLFH is indirectly associated with this factor. Clinical relevance of the identification of GLFH and HLO remains to be determined.

Abbreviations: BOAS, brachycephalic obstructive airway syndrome; FB, French Bulldogs; GI, gastrointestinal; GLFH, gastric lymphofollicular hyperplasia; HLOs, *Helicobacter*-like organisms; WSAVA, World Small Animal Veterinary Association.

KEYWORDS
bacterial, bacterial species, chronic gastritis, dog, gastric follicular hyperplasia, gastroenterology, *Helicobacter*, microbiology

1 | INTRODUCTION

Gastric lymphofollicular hyperplasia (GLFH) is characterized by the formation of lymphoid nodules and follicles in the gastric mucosa.[1] In humans, this finding may reflect active antigenic stimulation of gut-associated lymphoid tissue by *Helicobacter pylori*. Chronic *H. pylori* infection is suspected to activate follicular helper T-cells and lead to uncontrolled follicular B-cell proliferation.[1-3] These spiral bacteria predispose carriers to a high risk of developing gastric inflammation, gastric and duodenal ulcers, and low-grade lymphoma, of which the latter also has been reported in cats.[1-4]

Nevertheless, the pathophysiology of GLFH in dogs remains unknown. A recent study found an association between young age and GLFH in dogs.[5] In addition, brachycephalic dogs with increased inspiratory effort seem to be overrepresented in the population with GLFH.[5-7] Brachycephalic obstructive airway syndrome (BOAS) is known to be associated with a high prevalence of digestive disorders.[6] Surgical treatment of BOAS has been shown to improve digestive signs in approximately 90% of affected French Bulldogs (FBs).[6,7] One possible explanation for the occurrence and persistence of gastrointestinal (GI) signs in brachycephalic breeds may be related to possible associations among BOAS, GLFH, and *Helicobacter*-like organisms (HLOs).

The role of HLO infection in GLFH has not been investigated in dogs. In routine veterinary practice, HLOs are detected frequently on gastric histopathological assessments with special stains (Warthin-Starry).[8] Data suggest that HLOs could be observed in the superficial part of the gastric mucus, in the lumen of gastric glands and in the cytoplasm of parietal cells in dogs.[9,10] The presence of HLOs in the stomach is highly prevalent in healthy dogs, with 67% to 100% being carriers, and a higher prevalence has been reported with polymerase chain reaction (PCR) assessments.[11-13] These organisms colonize the gastric microbiota as early as 6 weeks of age.[9,14,15] In this context, treatment of HLO infection is extremely controversial. Assessment of clinical signs along with HLO infection score may help clinicians make therapeutic decisions.

To this end, our aims were to retrospectively evaluate the epidemiology, clinical signs, and endoscopic and histopathologic findings associated with GLFH, and evaluate their associations with HLOs and the FB breed in dogs with chronic GI signs.

2 | MATERIALS AND METHODS

2.1 | Case selection criteria

Electronic veterinary medical records at Fregis Veterinary Hospital for the period from January 2013 to January 2016 were reviewed to identify dogs with chronic (>3 weeks) GI signs that underwent upper GI endoscopy. Cases diagnosed with foreign body or neoplastic disease were excluded, as were those with incomplete clinical data, absence of GI endoscopy reports, or available gastric tissue for review.

2.2 | Medical record review

Data collected from the medical records included signalment (age, breed, sex, neuter status, and weight), clinical signs at admission (vomiting, regurgitation, diarrhea, cough, respiratory distress, and dysphagia), and whether surgical treatment (rhinoplasty and palatoplasty) was performed. Final diagnoses, treatments, and outcomes also were recorded when available. The study population was first divided into 2 groups: dogs with and without GLFH. These were then further categorized as the FB group (subgroup 1), non-FB brachycephalic dogs (subgroup 2), and the control group (all breeds excluding FB and brachycephalic dogs; subgroup 3). The breeds included in subgroup 2 included English Bulldog, Pug, Boston Terrier, Shih Tzu, Boxer, Dogue de Bordeaux, Cavalier King Charles Spaniel, and Brussels Griffon.

2.3 | Procedures

Each dog underwent upper GI endoscopy using a flexible video endoscope (EVIS EXERA II GIF-H180; Olympus America) and performed by a board-certified internist or senior resident. Endoscopic GI lesions were described and classified as normal, mild, moderate, marked, or severe, in accordance with the International GI Standardization Group (World Small Animal Veterinary Association) classification.[16] Specifically, gastric and duodenal lesions observed during endoscopy were described in terms of hyperemia, vascularity, edema, discoloration, friability, hemorrhage, erosion, ulceration, lacteal dilatation, texture, and contents. Gastrointestinal biopsy samples (minimum of 6 good quality samples from each region) were obtained for histological analysis as previously recommended.[17] A board-certified pathologist (LAPVSO, Toulouse, France) reviewed all of the biopsy samples. After dehydration, GI biopsy samples were embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin and Warthin-Starry stain. In accordance with WSAVA GI Standardization Group recommendations, gastric and duodenal lesions were scored using a 4-point grading system where 0 = microscopically normal, 1 = mild lesions, 2 = moderate lesions, and 3 = severe lesions.[17] The samples were examined under a light microscope using a 40× objective. Morphologic criteria included epithelial injury, fibrosis, glandular nesting, and

mucosal atrophy. Inflammatory variables included intraepithelial lymphocytes, lamina propria lymphocytes, plasma cells, eosinophils, and neutrophils. Diagnosis of GLFH was based on histopathology and scored according to the WSAVA standardization.[16,17] The presence of GLFH was scored separately between antral and body gastric mucosa, statistical analysis took into account both locations at the same time without combining them. Scores of GLFH were evaluated as follows: normal = small lymphoid aggregates or follicles occupying <10% of the biopsy area, mild = mild hyperplasia with lymphoid aggregates or follicles occupying 10% to 30% of the biopsy area, moderate hyperplasia = lymphoid aggregates or follicles occupying 30% to 50% of the biopsy area, and marked hyperplasia = lymphoid aggregates or follicles occupying >50% of the biopsy area (Figure 1). The Warthin-Starry stain was used to detect HLOs. Spiral and black HLOs were counted in 5 fields in each region, and the mean number of HLOs observed in each microscopic field was scored according to the following scale: 0 = no organisms observed, 1 = few organisms observed (1-20 organisms per field), 2 = moderate number of organisms observed (from 21 to 100 organisms per field), and 3 = high number of organisms observed (>100 organisms per field). For the duodenal samples, the evaluated histologic variables scored were villus stunting, epithelial injury, crypt distention, lacteal dilatation, mucosal fibrosis, intraepithelial lymphocytes, lamina propria lymphocytes and plasma cells, and the numbers of eosinophils and neutrophils.

2.4 | Statistical analysis

For descriptive statistics, continuous data were assessed for Gaussian distribution by histogram evaluation and the Shapiro-Wilk test (Gaussian if $P > .05$). Gaussian data were presented using mean (standard deviation [SD]) and non-Gaussian data were presented using median (minimum-maximum). Categorical data were presented as the number of dogs (percentage). Because histopathologic findings, including HLOs and GLFH scores, were provided at the gastric body and antrum locations, each dog had 2 scores for each histopathologic variable. Thus, the associations between GLFH and gastric location, signalment, previous BAOS surgery, clinical signs, and endoscopic and histopathologic findings were assessed using univariate mixed effects ordered logistic regression, with the individual dog being the random factor. A variable was considered a potential confounding factor when associated with both dependent and independent variables. This was confirmed by a change in the *P*-value of the independent variable when both the independent variable and the confounding factor were entered into the model. To identify confounding factors associated with HLO score or FB breed, the associations between HLO score and breed and gastric localization, signalment, previous BAOS surgery, clinical signs, and endoscopic and histopathologic findings were assessed using univariate mixed effects multinomial logistic regression, with the individual dog being the random factor. The probability of each HLO score depending on the age of the dog was predicted using predictive margins to depict the association between HLO score and age. Variables associated with both HLO score or breed and GLFH score ($P < .05$) in the univariate analysis were selected for the multivariate mixed effects ordered logistic regression model with follicular hyperplasia grading as the dependent variable and the individual dog as the random factor. Finally, the GLFH scores were compared among the 3 subgroups (FB, other brachycephalic dogs, and controls) by pairwise comparisons of marginal linear predictions. The impact of previous medication before endoscopy and histopathology was not analyzed. All statistical analyses were performed using commercially available software (STATA, version 14.0, StataCorp LP, College Station, TX) with a significance level set at $P < .05$. Figures were generated using GraphPad Prism v7.0 (GraphPad).

3 | RESULTS

3.1 | Demographic data

Three hundred and thirty cases were identified in the electronic veterinary medical database. Forty-two cases were excluded because of a final diagnosis of gastric foreign body or neoplasia, or because of incomplete data. The remaining 288 patients were included in the

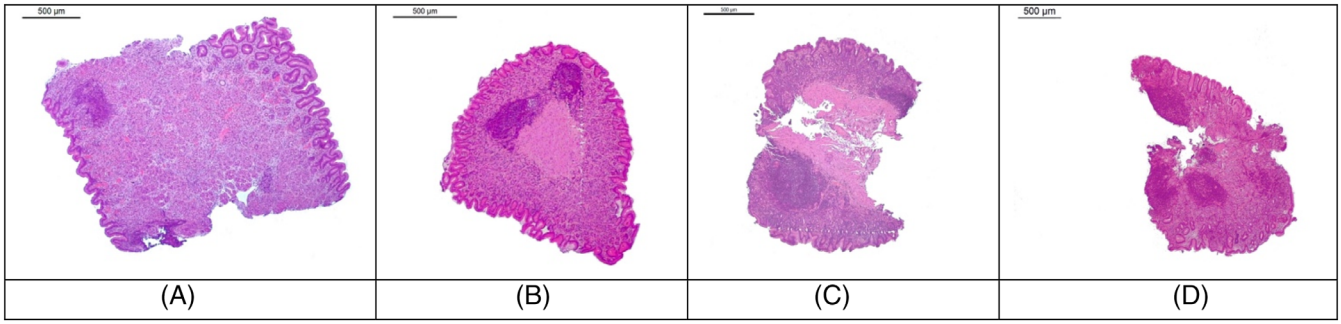

FIGURE 1 Histopathological scoring of lymphofollicular hyperplasia (LFH) of HES-stained gastric biopsies. (A) normal, small lymphoid aggregates or follicles occupying <10% of the biopsy area, (B) mild LFH, mild hyperplasia with lymphoid aggregates or follicles occupying 10%-30% of the biopsy area, (C) moderate LFH, lymphoid aggregates or follicles occupying 30%-50% of the biopsy area, and (D) marked LFH, lymphoid aggregates or follicles occupying >50% of the biopsy area

study. Of the included dogs, 143 (50%) were intact males, 19 (7%) were castrated males, 64 (22%) were intact females, and 61 (21%) were spayed females. The most prevalent breeds were FB (n = 63 [21.9%]), Yorkshire Terrier (n = 24 [8.3%]), mixed breed (n = 15 [5.2%]), Jack Russell Terrier (n = 13 [4.5%]), Golden Retriever (n = 12 [4.2%]), English bulldog (n = 11 [3.8%]), Chihuahua (n = 8 [2.8%]), Belgian Shepherd (n = 7 [2.4%]), Bernese Mountain (n = 5 [1.7%]), Cavalier King Charles Spaniel (n = 5 [1.7%]), Cocker Spaniel (n = 5 [1.7%]), Shih Tzu (n = 5 [1.7%]), and West Highland White Terrier (n = 5 [1.7%]). The median age at presentation was 5.0 years (range, 10 months to 15 years). The data for the 3 groups are presented in Table 1.

3.2 | Factors associated with the severity of GLFH

Gastric lymphofollicular hyperplasia was present in 138 of the 288 (48%) dogs, scored mild in 73 (53%), moderate in 48 (35%), and marked in 17 (12%) dogs. Age, sex, breed, and vomiting were significantly associated with GLFH (Table 2). Age showed an inverse association with GLFH severity ($P < .001$). Intact males were associated with GLFH ($P = .04$), with lower risk compared to intact female (OR, 0.28). In addition, FB ($P = .04$) and animals presented for vomiting ($P = .03$) showed more severe GLFH. Among the 138 dogs with GLFH, 80 (58%) presented with vomiting. No significant association was found between GLFH severity and weight, regurgitation, dysphagia, diarrhea, cough, respiratory distress, and BOAS surgery (Table 2). Among endoscopic variables, presence of discoloration ($P = .02$), ulceration ($P = .004$), and hemorrhage of the gastric mucosa ($P < .001$) during gastroscopy were statistically associated with GLFH severity. Forty-six (33%) dogs with GLFH had gastric ulcers. No significant association was observed between the presence of GLFH and gastric or duodenal hyperemia, edema, friability, duodenal discoloration, texture, hemorrhage, ulcers, lacteal dilatation, or duodenal content on upper GI endoscopy (Table S1). Finally, no difference in GLFH severity was found between the gastric body and antrum ($P = .8$).

On histological examination (Table 3), gastric lymphocytic infiltration of the lamina propria ($P < .001$), gastric intraepithelial lymphocytic infiltration (inverse association, $P = .004$), epithelial injury of the duodenum ($P = .04$), and HLO score ($P = .01$) were positively associated with the severity of GLFH. With regard to HLOs, 90/138 (66%) of the dogs with GLFH showed positive results. The HLO score and GLFH severity were significantly associated (a higher HLO score led to higher GLFH score and severity, $P = .01$). No association was found between gastric mucosal surface injury, gastric pit injury, presence of fibrosis or atrophy, eosinophilic or neutrophilic infiltration of the lamina propria, duodenal villus stunting, crypt distension, and the GLFH score.

3.3 | Factors associated with the HLO score

Helicobacter-like organisms were identified in 167/288 dogs (58%). Vomiting ($P = .01$) was associated with HLO score. An inverse association was observed between age and HLO score ($P < .001$). As depicted in Figure 2, the predicted probability of HLO score ≥1 was higher in younger dogs than in older animals.

Eighty percent of dogs (133/167) with HLOs presented with vomiting. Lymphocytic and plasmacytic infiltration of the lamina propria ($P = .03$) on histological examination was associated with the presence of HLOs. Ten (6%) of the HLO-infected dogs were treated with a combination of antibiotics: amoxicillin/clavulanic acid (12.5 mg/kg PO q12h), metronidazole (12.5 mg/kg PO q12h), and antiacid (omeprazole 1 mg/kg PO q12h). Follow-up data were available for 5 of these 10 dogs: 4 showed good resolution of clinical signs, whereas the other dogs did not improve after treatment.

3.4 | Factors associated with the FB breed

Gastric lymphofollicular hyperplasia was identified in 38/63 (60%) FBs, 21/45 (45%) non-FB brachycephalic dogs, and 79/180 (44%) dogs in the control group. Univariate analysis showed that young age ($P < .01$), intact male sex ($P = .01$), vomiting ($P = .02$), marked GLFH severity ($P = .04$), and HLO score 3 ($P < .001$) were statistically associated with the FB breed. Vomiting was more prevalent in FBs with GLFH (40/63; 63%) than in the overall population (86/180 dogs; 78%). The FB breed showed the highest GLFH score in comparison with the other 2 groups (Figure 3). No statistical difference was found in the severity of GLFH between the non-FB brachycephalic group (group 2) and the control group (group 3; $P = .21$) or between the FB and the non-FB brachycephalic groups ($P = .85$).

3.5 | Multivariate analysis of factors influencing the association between GLFH and the FB breed

Using multivariate analysis, age, vomiting, and HLO scores were found to be possible confounders for the association between GLFH and the

TABLE 1 Study group characteristics

Group	n	Median age (years; min-max)	Sex
1: French Bulldogs	63	2 (10-10 months)	47 IM, 3 CM, 9 IF, 4 SF
2: Non-FB brachycephalic dogs	45	4 (10-11 months)	22 IM, 3 CM, 11 IF, 9 SF
3: Control group	180	7 (10-15 months)	74 IM, 13 CM, 44 IF, 48 SF

Abbreviations: CM, castrated males; IF, intact females; IM, intact males; SF, spayed females.

TABLE 2 Univariate mixed-effect ordered logistic regression models of signalment and clinical independent variables associated with GLFH severity

Variable	% (total n/N-GLFH)	% (n/N-Non-GLFH)	Odds ratio (95% CI)	*P*-value
Intact female[a]	61 (39/64)	39 (25/64)	1.0	
Neutered female	53 (33/61)	47 (28/61)	0.41 (0.11-1.49)	.18
Intact male	47 (67/143)	53 (76/143)	0.28 (0.08-0.91)	.04
Neutered male	53 (10/19)	9 (9/19)	0.32 (0.01-5.23)	.45
Vomiting	55 (80/138)	45 (68/150)	3.36 (1.15-3.78)	.03
Regurgitation	22 (30/138)	14 (21/150)	1.52 (0-35-6.53)	.58
Diarrhea	23 (32/138)	44 (66/150)	0.74 (0.23-2.29)	.6
Cough	6 (8/138)	9 (13/150)	0.31 (0.04-2.01)	.22
Respiratory distress	22 (30/138)	18 (27/150)	1.83 (0.34-9.50)	.48
Dysphagia	18 (25/138)	25 (37/150)	0.72 (0.14-3.50)	.69
Group 1: French Bulldogs	60 (38/63)	40 (25/63)	3.81 (1.0-13.53)	.04
Group 2: Non-FB brachycephalic dogs	46 (21/45)	53 (24/45)	3.14 (0.53-18.59)	.21
Group 3: Control group[b]	44 (80/180)	56 (100/180)	1.0	

Abbreviations: GLFH, gastric lymphofollicular hyperplasia; HLOs, *Helicobacter*-like organisms; N, GLFH; total population with GLFH; n/N-Non-GLFH: percentage of dogs with the variable and without GLFH in all dogs free of GLFH Total n/N-GLFH: percentage of dogs with the variable and GLFH in all dogs with GLFH.

[a]Reference category: intact females.

[b]Reference category: control group.

FB breed. The HLO scores were positively associated with GLFH ($P < .001$), and high HLO scores were significantly associated with high GLFH score and severity (Figure 4). Gastric lymphofollicular hyperplasia still was associated with vomiting ($P = .01$). However, GLFH was no longer statistically associated with the FB breed ($P = .76$) and young age ($P = .11$; Table 4).

4 | DISCUSSION

We showed a positive association between GLFH and HLOs in dogs with chronic GI signs. *Helicobacter*-like organisms were more prevalent in young dogs. In multivariate analysis, neither GLFH nor HLO scores were statistically associated with the FB breed.

A positive association between GLFH and HLO scores on histology has been reported in humans and cats.[1-4,18] However, previous studies using dogs have not identified a clear link between chronic gastritis and the presence of HLOs.[19,20] One possible reason for this lack of association is the method of detection of HLOs, because most of the studies performed PCR on gastric biopsy samples. Despite its excellent sensitivity and specificity, both of which have been reported to be 100% in humans, the positive predictive value of this test is low because of the high prevalence of HLOs in healthy and vomiting dogs (67%-100%).[21,22] However, our results showed a positive association between GLFH and HLOs based on histologic quantification. A higher score of HLOs was associated with a higher score and severity of GLFH, as shown in Figure 3 and Table 4. The large number of cases, the positive association, and the increased OR (21.6) for dogs with a high score for organisms highlighted the evidence of a link between GLFH and HLOs. In humans, *H. pylori* is 2.3 times more prevalent in patients with GLFH than in those without GLFH.[1,23] The presence of these bacteria in the stomach induces the production of proinflammatory cytokines, such as interleukin 12, stimulating Th1 lymphocytes to form lymphoid follicles.[18] Moreover, in humans, the presence of *H. pylori* in the stomach may influence the gut microbiota by decreasing its diversity and inducing dysbiosis, contributing to chronic inflammation.[18,21,24] According to a previous study, these factors associated with chronic inflammation could explain the close relationship between GLFH and gastric lymphoma.[1] In dogs, information about the immune response to *Helicobacter* spp. is scarce.[11] In our study, all GI cancers were excluded, and thus we could not evaluate the association between HLOs and GI neoplasia. However, in cats, depending on the *Helicobacter* spp., an association with chronic gastritis, gastric lymphoma, or even zoonosis, in exceptional cases, has been reported.[4,11,25]

With regard to the epidemiological findings in our study, intact males were less affected by GLFH than were intact females. This finding could have been confounded by the majority of intact males (47/63) in the FB group, which corresponded to 21% of the entire population. However, previous studies have shown that males with more pronounced BOAS might show more severe clinical signs and more severe gastritis than females with BOAS.[6,26] Additional studies are needed to determine whether intact males can be subclinical carriers of HLOs, or whether this result is an outlier.

In our study, young age was associated with higher HLO and GLFH scores. The results showed that the GLFH score was higher in young dogs with a higher density of HLOs. As already observed in children, the presence of HLOs and high score were associated with

TABLE 3 Univariate mixed-effect ordered logistic regression models of independent histopathological variables associated with GLFH severity

Variables	Grade	% total n/N-GLFH	% n/N-Non-GLFH	Odds ratio (CI 95%)	*P*-value
Surface injury	0[a]	48 (67/138)	47 (71/150)	1.0	
	1	32 (44/138)	29 (42/150)	0.65 (0.26-1.59)	.35
	2	17 (23/138)	17 (26/150)	0.81 (0.24-2.68)	.73
	3	3 (4/138)	7 (11/150)	0.16 (0.01-2.34)	.18
Gastric pit injury	0[a]	57 (80/138)	56 (86/150)	1.0	
	1	31 (42/138)	29 (43/150)	0.57 (0.24-1.29)	.18
	2	9 (13/138)	9 (13/150)	0.80 (0.22-2.84)	.73
	3	2 (3/138)	5 (8/150)	0.13 (0.01-2.09)	.15
Gastric fibrosis atrophy	0[a]	55 (77/138)	51 (77/150)	1.0	
	1	24 (34/138)	34 (52/150)	0.87 (0.037-2.01)	.75
	2	18 (26/138)	13 (19/150)	0.53 (0.17-1.63)	.27
	3	2 (2/138)	2 (2.4/150)	0.58 (0.057-5.82)	.64
Gastric intraepithelial lymphocyte	0[a]	55 (76/138)	49 (73/150)	1.0	
	1	38 (53/138)	46 (70/150)	0.06 (0.01-0.41)	.004
	2	5 (7/138)	4 (6/150)	0.47 (0.03-5.97)	.56
	3	1 (2/138)	1 (1/150)	3.03 (0.04-191.19)	.6
Gastric lamina propria lymphocytes	0[a]	4 (6/138)	15 (22/150)	1.0	
	1	44 (75/138)	48 (71/150)	3.23 (0.94-11.10)	.06
	2	45 (62/138)	32 (49/150)	10.68 (3.09-36.86)	<.001
	3	7 (10/138)	5 (8/138)	71.36 (8.65-588.46)	<.001
Gastric lamina propria eosinophils	0[a]	91 (127/138)	87 (131/150)	1.0	
	1	6 (8/138)	10 (14/150)	1.57 (0.033-7.44)	.57
	2	2 (4/138)	2 (4/150)	1.42 (0.09-21.50)	.8
	3	0 (0/138)	1 (1/150)	0.01 (0)	1.0
Gastric lamina propria neutrophils	0[a]	86 (118/138)	84 (126/150)	1.0	
	1	14 (20/138)	16 (24/150)	0.27 (0.01-5.93)	.63
	2	0 (0/138)	0 (0/138)	0.01 (0)	1.0
Helicobacter spp.	0[a]	34 (48/138)	48 (73/150)	1.0	
	1	26 (36/138)	18 (28/150)	7.88 (2.66-23.25)	<.001
	2	22 (31/138)	16 (25/150)	5.71 (1.38-23.58)	.02
	3	17 (23/138)	9 (14/150)	21.66 (2.30-203.97)	.01
Duodenal villus stunting	0[a]	82 (114/138)	78 (116/150)	1.0	
	1	13 (18/138)	18 (27/150)	0.58 (0.13-2.52)	.5
	2	4 (8/138)	3 (5/150)	1.49 (0.06-32.74)	.8
	3	0 (0/138)	1 (2/150)	0.01 (0)	1.0
	0[a]	92 (127/138)	83 (125/150)	1.0	
Duodenal epithelial injury	1	8 (11/138)	11 (17/150)	0.14 (0.02-0.91)	.04
	2	5 (6/138)	4 (6/150)	0.19 (0.13-2.64)	.2
	3	1 (2/138)	2 (3/150)	0.43 (0.01-16.09)	.65
Duodenal crypt distension	0[a]	88 (122/138)	87 (131/150)	1.0	
	1	9 (12/138)	9 (13/150)	0.34 (0.06-1.90)	.22
	2	2 (3/138)	3 (4/150)	0.27 (0.01-5.82)	.4
	3	1 (1/138)	1 (1/50)	0.04 (0.01-3.66)	.16
Duodenal lacteal distension	0[a]	68 (94/138)	66 (99/150)	1.0	
	1	22 (30/138)	18 (27/150)	0.85 (0.25-2.85)	.8
	2	10 (14/138)	8 (12/150)	0.66 (0.12-3.43)	.62
	3	1 (1/138)	0 (0/150)	0.76 (0.01-1972.48)	.94

TABLE 3 (Continued)

Variables	Grade	% total n/N-GLFH	% n/N-Non-GLFH	Odds ratio (CI 95%)	*P*-value
Duodenal fibrosis	0[a]	90 (125/138)	88 (133/150)		
	1	8 (11/138)	7 (11/150)	0.98 (0.17-5.66)	.98
	2	1 (2/138)	1 (2/150)	0.3 (0.01-15.38)	.55
	3	1 (1/138)	1 (1/150)	0.07 (0.01-18.21)	.34
Duodenal intraepithelial lymphocytes	0[a]	88 (120/138)	89 (134/150)	1.0	
	1	6 (8/138)	9 (14/150)	0.15 (0.01-1.10)	.06
	2	2 (3/138)	2 (3/150)	1.41 (0.01-138.52)	.88
	3	0 (0/138)	0 (0/150)	0.01 (0)	1.0
Duodenal lamina propria lympocytes	0[a]	20 (29/138)	33 (50/150)	0.1	
	1	22 (41/138)	19 (28/150)	0.96 (0.01-4.60)	.95
	2	51 (70/138)	42 (63/150)	1.21 (0.28-5.06)	.8
	3	8 (12/138)	6 (9/150)	0.83 (0.57-12.16)	.9
Duodenal lamina propria eosinophils	0[a]	90 (125/138)	89 (135/150)	1.0	
	1	7 (9/138)	8 (12/150)	0.46 (0.07-2.80)	.4
	2	2 (3/138)	2 (2/150)	0.21 (0.08-5.44)	.35
	3	1 (1/138)	1 (1/150)	0.28 (0.01-134.54)	.69
Duodenal lamina propria neutrophils	0[a]	82 (113/138)	86 (129/150)	1.0	
	1	15 (20/138)	11 (15/150)	0.05 (0.01-0.50)	.85
	2	3 (4/138)	2 (3/150)	2.94 (2.16-3.70)	.46
	3	0 (0/138)	1 (1/150)	6.36 (5.35-7.91)	.25

Abbreviations: CI, confidence interval; GLFH, gastric lymphofollicular hyperplasia; N, GLFH; total population with GLFH; n/N-Non-GLFH, percentage of dogs with the variable and without GLFH in all dogs free of GLFH; Total n/N-GLFH, percentage of dogs with the variable and GLFH in all dogs with GLFH.
[a]Reference category.

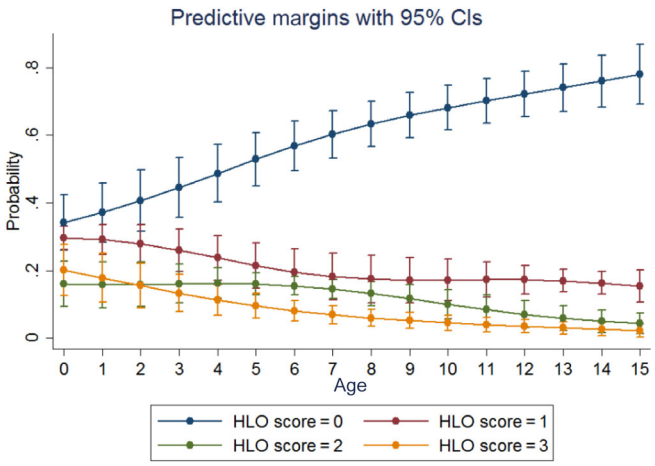

FIGURE 2 Predictive margins of the probability of each HLO score (on the y-axis) depending on the age of the dog (on the x-axis). The predicted probability of an HLO score of 0 was much lower in a 1-year-old dog (.37) than in a 15-year-old dog (.78). Error bars represent 95% confidence intervals. HLOs, *Helicobacter*-like organisms

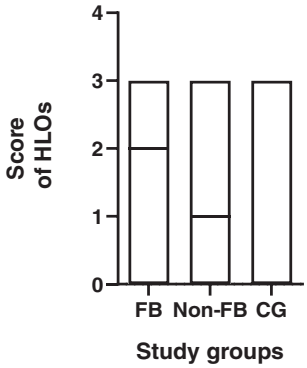

FIGURE 3 Comparison of HLO scores among the 3 groups. The FB breed showed the highest HLOs score comparing to non-French Bulldog brachycephalic dogs and control group ($P < .001$, confidence interval 95%). The line represents the median HLOs score for each group. Control group (CG) had a median of 0. Boxes represent the minimum and maximum score for each group. Score 0 = no HLOs observed, 1 = few organisms observed (1-20 organisms per field), 2 = moderate number of HLOs observed (from 21 to 100 organisms per field), and 3 = high number of HLOs observed (>100 organisms per field). CG: control group; FB, French Bulldog; HLOs, *Helicobacter*-like organisms; Non-FB, non-French Bulldog brachycephalic dogs

higher scores of GLFH in young dogs.[1,18] One interesting study found that adult mice infected with virulent *Helicobacter* spp. developed preneoplastic lesions within 2 to 4 months, whereas neonatal mice showed an immune tolerance to the pathogen. Therefore, *Helicobacter* spp. could be beneficial for the host when present in the stomach at a young age. On the basis of this finding, we hypothesized that the development of GLFH in children could be a part of the normal maturation of gastric immunity.[2] If considered part of the normal gastric

microbiota, this hypothesis could explain why young dogs had high HLO scores in our study. If so, the implementation of treatment in young dogs could be questionable.

In our study, GLFH and HLOs showed a direct association with vomiting (58% and 80%, respectively). However, because of the presence of concomitant inflammatory infiltrates, it is difficult to conclude that the presence of GLFH or HLOs was the only cause of vomiting. In fact, in our study, animals without GLFH also showed high rates of vomiting (45%, 68/150). Finally, our endoscopic and histological results showed an association between GLFH and gastric ulcers. The chronic inflammation created by HLOs and the lymphocytic immune response of the gastric mucosa could cause gastric ulcers. In humans, GLFH has been associated with the presence of gastric and duodenal ulcers in 36% and 41% of cases, respectively.[23]

In humans, HLO-specific treatment has been shown to resolve GLFH, but the use of this form of treatment in veterinary medicine has been a topic of debate.[27,28] Despite the reported link between GLFH and HLOs in cases of vomiting, clinicians should consider other causes of chronic GI signs before incriminating HLOs.[12,19,20,29] In our study, only 6% of the patients infected by HLOs underwent an antibiotic treatment trial because of the lack of satisfactory improvement with a 50% response rate.

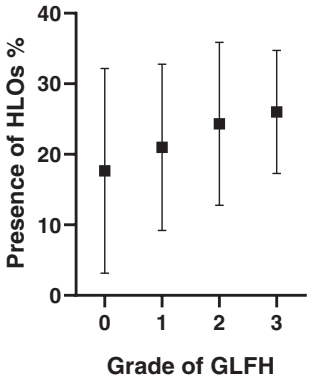

FIGURE 4 Positive association between the GLFH grade and the presence of HLOs. Positive association between the GLFH grade and the presence of HLOs in the global population ($P < .001$). GLFH, gastric lymphofollicular hyperplasia; HLOs, *Helicobacter*-like organisms. Error bars represent 95% confidence intervals

Our last aim was to evaluate the association between the FB breed and GLFH. According to our experience and a previous study, FBs seemed to be predisposed to GLFH. We believe that this apparent association is circumstantial[5] because we usually evaluate FBs at a young age to evaluate BOAS or because they are experiencing vomiting or regurgitation. In these young dogs, HLO infection is highly prevalent. The HLO score was statistically associated with the GLFH score (grades 2 and 3). One possible explanation is the anatomical brachycephalic conformation of the upper respiratory tract of FBs, which makes them more sensitive to the development of chronic gastritis at a young age with more severe clinical signs and endoscopic and histologic findings.[6,7,26] Interestingly, no significant differences in clinical signs were observed between the non-FB brachycephalic group and the control group in our study. This explanation remains hypothetical and needs to be further evaluated by following up FBs at a later stage of their lives.

The main limitations of our study were its retrospective nature and the method used to diagnose the presence of HLOs. Because ours was a retrospective study, the groups were not randomized or standardized. The treatments administered before presentation were not taken into account. Some dogs had received antacid (omeprazole) and antibiotic treatment (metronidazole or tylosine) to rule out antibiotic-responsive enteropathy. These treatments could have influenced the prevalence of HLOs (58% of the entire population), which was lower than that reported in previous studies from Europe (80% in Germany, 87% in Portugal).[19,20] The sensitivity of histopathological analysis depends on the number, site, and size of the biopsy samples acquired, and biopsy samples from the greater curvature and body of the stomach can increase the accuracy of HLO diagnosis.[8,22] Despite the distinctive morphology of *Helicobacter* spp., histopathological score results may be influenced by factors such as presence of patchy colonization, bacteria cross sections, other non-*Helicobacter*

TABLE 4 Multivariate mixed-effect ordered logistic regression of variables associated with GLFH

Variables	Group/grade	Total n/N-GLFH (%)	n/N-Non-GLFH (%)	Odds ratio (CI 95%)	*P*-value
Breed	1	60 (38/63)	40 (25/63)	1.22 (0.33-4.42)	.76
	2	45 (21/45)	54 (24/45)	1.75 (0.28-10.83)	.55
	3[a]	44 (80/180)	56 (100/180)		
Age				0.89 (0.77-1.02)	.11
Vomiting	1	55 (80/138)	45 (68/150)	4 (1.37-11.70)	.01
HLO	0[a]	34 (48/138)	48 (73/150)	1.0	
	1	26 (36/138)	18 (28/150)	5.87 (2.22-15.52)	<.001
	2	22 (31/138)	16 (25/150)	5.99 (2.07-17.29)	<.001
	3	17 (23/138)	9 (14/150)	16.71 (4.36-63.91)	<.001

Abbreviations: CI, confidence interval; FB, French Bulldog; GLFH, gastric lymphofollicular hyperplasia; Group 1, French Bulldogs; Group 2, non-FB brachycephalic dogs; Group 3, control group; HLOs, *Helicobacter*-like organisms; N, GLFH; total population with GLFH; n/N-Non-GLFH: percentage of dogs with the variable and without GLFH in all dogs free of GLFH; Total n/N-GLFH: percentage of dogs with the variable and GLFH in all dogs with GLFH.
[a]Reference category.

spp. such as *Pseudomonas fluorescence* having similar morphology and causing a mistaken diagnosis.[8,19] The gold standard test for the diagnosis of HLO infection remains PCR on gastric biopsy samples.[22] In our study, we diagnosed HLOs on the basis of histopathological identification and scoring by subjective quantification, as has been previously reported in humans.[30] Future studies on endoscopic and histologic follow-up during their lives should make it possible to determine whether the presence of HLOs in the stomach is a normal developmental finding in dogs.

In conclusion, we showed that HLOs were more prevalent in young dogs and that high HLO scores were statistically associated with higher GLFH score and severity. Additional studies are needed to determine if infection by HLOs and the appearance of GLFH are normal steps in gastric immunity maturation.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

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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