



Proteomic Characterization of Canine Gastric Fluid by Liquid Chromatography–Mass Spectrometry for Development of Protein Biomarkers in Regurgitation, Vomiting, and Cough

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Reflux and aspiration in people cause and exacerbate respiratory diseases in the absence of gastrointestinal signs. Protein biomarkers in humans detect extraesophageal reflux (EER) from oropharyngeal (OP) and bronchoalveolar lavage samples. Reflux likely contributes to respiratory disease in dogs. The objectives of this study were to analyze the canine gastric fluid (GF) proteome and compare this to the OP proteome in normal, vomiting/regurgitating, and coughing dogs to identify biomarkers for EER/aspiration. Twenty-three client-owned dogs were enrolled. Canine GF samples ($n = 5$) and OP swabs in normal ($n = 6$), vomiting/regurgitating ($n = 7$), and coughing ($n = 5$) dogs were within 2 weeks of sample collection. Protein digests were analyzed by liquid chromatography–mass spectrometry. Differential abundance (DA) of proteins between groups was evaluated by Fisher's exact test with $p < 0.0004$ significance level after correction for multiple comparisons. DA was found between all groups ($p < 0.0001$): GF vs. normal ($n = 130$ proteins), coughing vs. normal ($n = 22$ proteins), and vomiting/regurgitating vs. normal ($n = 20$ proteins). Protein abundance was highly variable between dogs. Gastrointestinal-specific proteins were found in OP swabs from vomiting/regurgitating and coughing dogs but not from healthy dogs. In conclusion, the proteomic composition of the OP varies between health and disease. The presence of gastrointestinal-specific proteins in OP of coughing dogs may suggest reflux and/or aspiration as contributing factors. The variable protein abundance warrants investigation into biomarker panels.

Keywords: reflux, aspiration, extra-esophageal, oropharyngeal, respiratory

INTRODUCTION

Biomarkers diagnose, monitor, and identify populations at risk for disease. Biomarkers also have the potential to advance the understanding of disease pathogenesis by allowing an objective investigation of the efficacy of novel therapeutics (1–5). In recent years, the development and implementation of biomarkers have been making an increasingly large impact in companion animals (2, 5).

Reflux of gastrointestinal contents is a source of acute and chronic pulmonary disease, with a prevalence of 50% in humans with chronic cough, asthma, chronic obstructive pulmonary disease, and idiopathic pulmonary fibrosis (6–10). Reflux and aspiration are implicated in canine respiratory disorders including rhinitis, laryngeal dysfunction, and upper/lower airway and pulmonary parenchymal diseases (11–13). Despite the implied association between reflux, aspiration, and respiratory disease, a non-invasive widely available means of detecting reflux and aspiration in dogs is needed.

The two major types of reflux implicated in the development of respiratory disease are gastroesophageal reflux (GER) and extraesophageal reflux (EER). Gastroesophageal reflux refers to reflux of gastric contents into the esophagus, while EER refers to refluxate reaching structures beyond the esophagus, including the oro- and nasopharynx, larynx, and airways (14). Reflux reaching the nasopharynx and larynx are referred to as nasopharyngeal reflux and laryngopharyngeal reflux, respectively. The severity of GER and the prevalence of EER are correlated (15). In people, EER increases the risk of macro- and microaspiration, laryngeal dysfunction, and exacerbation of pulmonary pathology (14, 15). Importantly, treatment of EER in people reduces the frequency of respiratory disease exacerbation and slows the rate of decline in lung function (7). This makes identifying patients with reflux-associated respiratory disease critically important.

Human gastric fluid (GF) has been extensively characterized, allowing for the identification of reflux biomarkers outside the gut (16–18). Gastric pepsin (pepsin A), a component of refluxate identified in humans with EER, is a biomarker in oral and respiratory secretions with improved sensitivity (100%) over ambulatory esophageal pH monitoring (63%) (16, 18–21). Though proteomic evaluation has been performed for several biological fluids in dogs (3, 22), characterization of the canine GF proteome has not been performed previously.

The objectives of this pilot study were to characterize the protein composition of canine GF using liquid chromatography–mass spectrometry (LC–MS) and compare the GF proteomic profile to that of the OP in healthy, vomiting/regurgitating, and coughing dogs to detect potential biomarkers of EER. We hypothesized that by using a high-coverage proteome analysis we could identify proteins relevant as potential biomarkers of canine extraesophageal reflux disease (EERD).

MATERIALS AND METHODS

Animals

Twenty-three companion dogs presenting to the University of Missouri Veterinary Health Center were prospectively enrolled with informed owner consent (University of Missouri IACUC #9154). Residual canine GF was collected directly *via* nasogastric or orogastric tubes in patients for whom these interventions were deemed medically necessary by the attending clinician. Sampling of gastric fluid from healthy dogs could not be ethically justified due to risk to the patient. Gastric fluid samples were collected prior to feeding (≥ 6 h). Oropharyngeal swabs were collected from healthy dogs, dogs with a history of regurgitation/vomiting, and coughing dogs. The healthy dogs had an unremarkable

physical examination and no clinical evidence of gastrointestinal (GI) or respiratory disease within the preceding 6 months. The regurgitating/vomiting dogs were enrolled if they had a documented episode of regurgitation or vomiting within 12 h of sample collection. The coughing dogs were enrolled if they had a history of cough for >2 weeks without a clinical evidence of GI disease (e.g., vomiting and regurgitation). Dogs were excluded from the study if they had a mixed clinical evidence of respiratory and GI disease or if they were receiving prokinetics, antacids, antibiotics, or probiotics at least 2 weeks before the sample collection. Dogs with a gag at the end of a cough paroxysm were not considered to have a GI disease and were therefore not excluded.

Sample Collection

Two milliliters of GF was collected *via* nasogastric or orogastric tubes and placed in a sterile red top blood collection tube. Samples were collected from unsedated dogs using minimum physical restraint needed for sample collection. Oropharyngeal swabs, collected by vigorously rubbing this region in each gently restrained dog meeting our inclusion criteria, were placed in 2 ml of sterile saline in a red top tube as mentioned earlier. The samples were gently vortexed and stored in polypropylene conical bottom Eppendorf tubes (Fischer Scientific, Chicago, IL) at -20°C . All samples were evaluated within 2 weeks of collection.

Proteome Analysis

Proteome analysis was performed by the Gehrke Proteomics Center at the University of Missouri. Protein was extracted using four volumes of ice-cold acetone. Following washing with 80% acetone (in water), the pellet was re-suspended with 6 M urea, 2 M thiourea, and 100 mM ammonium bicarbonate. Protein was quantified using the EZQ assay according to the manufacturer's instructions (Invitrogen/Life Technologies). After quantification, the protein amount was normalized across all samples by dilution in urea buffer. Twenty-five micrograms of protein from each sample was digested with trypsin. The resulting peptides were desalted using C18 pipette tips (Pierce/Thermo), lyophilized, and re-suspended in 25 μl of acetonitrile/formic acid (0.1%). Ten micrograms of the re-suspended peptides was loaded on a C8 trap column (Thermo PepMap 100, 5 cm \times 300 μm) and then separated using a 400-nl/min 70-min gradient on a self-packed C18 column (75 μm \times 20 cm \times 1.7 μm particles—Waters BEH C18) at 50°C in the CaptiveSpray nanospray source. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), and the LC gradient was for B: initially 2%, followed by 26-min ramp up to 17%, 17–25% over 36 min, 25–37% over 15 min, gradient of 37–80% over 6 min, and then held at 80% for 7 min, with a total run time of 90 min. MS + MS/MS data were acquired on a Proxeon Easy nLC system attached to an LTQ Orbitrap mass spectrometer using the parallel accumulation serial fragmentation (PASEF) (1) method over the 90-min gradient. Capillary voltage was set to 1,600 V, trapped ion mobility spectrometry (tims)-on, PASEF-on (10 PASEF frames, overlap of five; i.e., between MS acquisitions), and 100–1,700 m/z mass range. Cycle time was for 1 MS and 15 PASEF = 1.8 s (~ 120

MS/MS acquired per cycle). Repeated acquisition was threshold $\times 4$ within 0.4 min. Active exclusion involved release after 1 min (exclude from MS/MS the same peptide mass, notwithstanding the criteria above, and release after 1 min of elution). MS data were collected over an m/z range of 100–1,700. During MS/MS data collection, each time cycle included 1 MS + an average of 10 PASEF MS/MS scans.

Data Analysis

The LTQ Orbitrap XL raw files were submitted to the Proteome Discoverer search engine for protein identification (V2.2.0.388; Sequest HT). The protein database for *Canis lupis familiaris* (50,035; last update 2/5/2019 sequences) was downloaded from NCBI using the search term “dogs.” Data were searched with the following parameters: trypsin (full) as enzyme, two missed cleavages allowed, six- to 150-amino-acid peptide length, 10 ppm mass tolerance on precursor ions, 0.6 Da on fragment ions, carbamidomethyl cysteine as a fixed modification, and oxidized methionine as variable. Spectrum grouper was used with the following parameters: precursor mass criterion, “same measure mass-to-charge”; precursor mass tolerance, 10 ppm; max RT difference (min), 1.5; allow mass analyzer mismatch, false; and allow MS order mismatch, false. The Percolator algorithm was used to automatically search a decoy database with the following criteria: target false discover rate (FDR, strict) 0.01, validation based on q -value. Proteins that passed this “high” confidence cutoff were examined further. Differential abundance (DA) of proteins was determined using PEAKS label-free quantitation based on the MS1 peak integration approach with the following parameters: algorithm: PEAKS-Q, retention time tolerance: 1 min, and mass tolerance: 40 ppm. Data were then filtered for 1% protein FDR with ≥ 1 unique peptide and ≥ 2 -fold change. Spectral counting was used to determine differential protein abundance. Data were filtered for 1% protein FDR with ≥ 1 unique peptide and then > 4 spectral counts per protein (mean of replicates). Protein abundance was described using the normalized spectral abundance factor (NSAF). Biological function was determined for the 200 most abundant proteins per group (GF, normal, vomiting/regurgitating, and coughing) as determined by NSAF. Biomarker candidates were evaluated according to their abundance in GF, tissue specificity, low or absent concentration in normal dog OP, and increased concentration in vomiting/regurgitating compared to normal dog OP. Candidate proteins were then assessed in coughing dogs to look for an evidence of EERD.

Statistical Analysis

Statistical analysis was performed using Past (version 3.21) and SigmaPlot (version 14.0) data analysis software. Descriptive statistics were applied where appropriate as median (interquartile range, IQR). Differences in patient demographics between groups (GF, healthy, vomiting/regurgitating, and cough) were evaluated by Wilcoxon signed rank test with $p \leq 0.05$ significance level. Principal component analysis was performed for all proteins identified across groups. Differential abundance of proteins between groups based on pairwise comparisons was evaluated by Fisher’s exact test, with $p < 0.0004$ considered significant after

TABLE 1 | Demographic data (age, weight, body condition score, sex and reproductive status) for each group are displayed as median (IQR).

	Healthy (<i>n</i> = 6)	Coughing (<i>n</i> = 5)	Vomiting/ regurgitating (<i>n</i> = 7)	Gastric fluid (<i>n</i> = 5)
Age (years)	8.5 (7–12.3)	7 (3.5–10)	9 (8–10)	9 (9–10)
Weight (kg)	15.4 (11.2–16.5)	20 (4.5–26.2)	10.3 (9–25.5)	20.3 (18.9–33)
Body condition score (nine-point scale)	5.5 (5–6.25)	5 (4–5)	5 (4.5–6.5)	6 (5–6)
Intact females	0	0	2	0
Intact males	0	1	1	0
Spayed females	4	2	3	3
Castrated males	2	2	1	2

Sex and reproductive status are also reported for each group. No statistically significant differences were detected between groups ($p > 0.05$).

correction for multiple comparisons. Coefficients of variation were calculated for each protein demonstrating a differential abundance between groups. A one-way analysis of similarity (ANOSIM) using the Bray–Curtis similarity index was used to test for similarity between groups based on the biological function of the detected proteins with $p \leq 0.001$ significance level. Normality was evaluated by Shapiro–Wilk test. *Post-hoc* analysis (Dunn’s method or Bonferroni correction for multiple comparisons) was applied where appropriate.

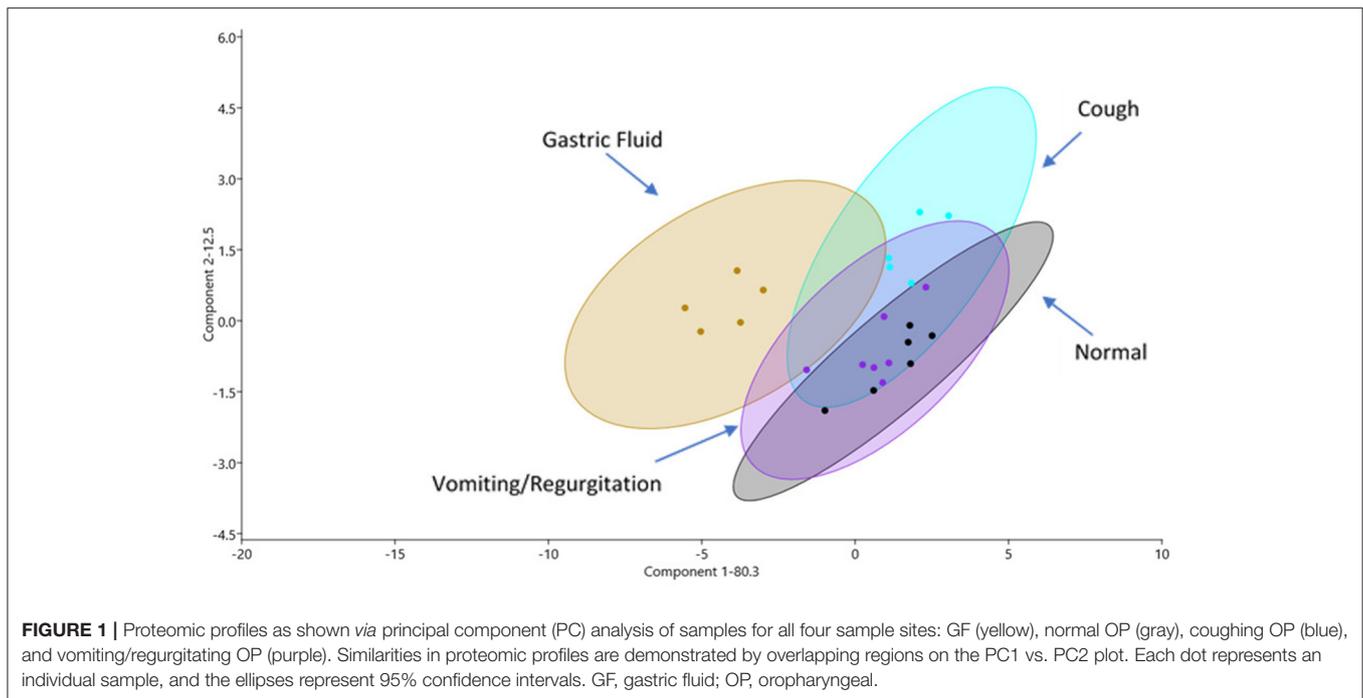
RESULTS

Animals

Twenty-three companion dogs were prospectively enrolled. The breeds represented included mixed breed ($n = 5$), Labrador retriever ($n = 3$), Yorkshire terrier ($n = 2$), standard poodle ($n = 2$), and one each for Australian shepherd, Welsh corgi, basset hound, Jack Russel terrier, beagle dog, golden retriever, German Shepherd dog, Boston terrier, bull terrier, miniature poodle, and Catahoula leopard dog. Twelve dogs were spayed females, seven were castrated males, two were intact males, and two were intact females. No significant differences were detected between groups [GF ($n = 5$), healthy ($n = 6$), vomiting/regurgitating ($n = 7$), and coughing ($n = 5$)] for age, weight, or body condition score (BCS) (Table 1). These results were therefore grouped and are provided as range and median (IQR). The ages ranged from 6 months to 13 years, with a median (IQR) age of 9 years (7–10 years). The weights ranged from 1.7 to 43 kg, with a median (IQR) weight of 16.6 kg (9–29 kg). The body condition score (nine-point scale) ranged from 4 to 8, with a median (IQR) BCS of 5 (5–6) (23).

Proteomic Analysis

From all samples, a total of 504 individual proteins were identified (Supplementary Materials). Within-group evaluation showed no significant differences for the total number of proteins identified. The median (IQR) number of proteins (per sample) per group were as follows: GF—122 proteins (110–230),



healthy—259 proteins (203–309), vomiting/regurgitating—231 proteins (203–240), and cough—258 proteins (254–325). No significant differences between groups were found for the total number of proteins identified. The principal component analysis demonstrated an overlap between GF and OP swabs from vomiting/regurgitating and coughing dogs but not from normal dogs (**Figure 1**). Three pairwise comparisons were performed to identify DA proteins between groups: (1) normal vs. GF, (2) normal vs. vomiting/regurgitating, and (3) normal vs. cough.

Normal vs. GF

One hundred thirty proteins showed a significant DA ($p < 0.0004$). Seventy proteins were significantly greater in abundance in GF; 60 were significantly greater in OP swabs from normal dogs. The coefficients of variation between dogs ranged from 16.9 to 244.9 for the DA proteins.

Normal vs. Vomiting/Regurgitating

Twenty proteins showed a significant DA ($p < 0.0004$). Thirteen proteins were significantly greater in abundance in OP swabs from vomiting/regurgitating dogs; seven were significantly greater in OP swabs from normal dogs. The coefficients of variation between dogs ranged from 45.5 to 264.6 for the DA proteins.

Normal vs. Cough

Twenty-two proteins showed a significant differential abundance ($p < 0.0004$). Twelve proteins were significantly greater in abundance in OP swabs from coughing dogs; 10 were significantly greater in OP swabs from normal dogs. The coefficients of variation between dogs ranged from 41.7 to 244.9 for DA proteins.

The OP proteome was also evaluated for GI-specific proteins. Gastrointestinal-specific proteins were defined as proteins that

showed tissue specificity to the stomach, intestines, or pancreas. GI-specific proteins were found on OP swab in 0/6, 2/5, and 7/7 healthy, coughing, and regurgitating/vomiting dogs, respectively. Of the GI-specific proteins detected in OP, 4/6 were pancreatic in origin.

For the biological function of proteins, all groups were found to be dissimilar on ANOSIM, with $p \leq 0.05$ (**Figure 2**). The principal component analysis demonstrated an overlap between GF and OP swabs from vomiting/regurgitating and coughing dogs. No overlap was found between normal and vomiting/regurgitating dogs (**Figure 3**). The metabolic and immune proteins were over-represented in GF compared to normal dog OP swabs ($p \leq 0.05$). Seventy-three percent of gastric metabolic proteins were pancreatic in origin, which is over-represented compared to salivary (14%), gastric (10%), and intestinal (3%) proteins ($p \leq 0.05$). Pancreatic metabolic proteins were also increased in overall abundance compared to salivary, gastric, and intestinal metabolic proteins by NSAF. Pepsin A was identified in GF from all dogs but was less abundant compared to other non-gastric metabolic proteins (**Table 2**). Metabolic proteins were likewise over-represented in OP swabs in vomiting/regurgitating dogs compared to healthy dogs ($p < 0.05$) as were proteins with an immunologic function ($p < 0.05$). The coughing dogs had increased numbers of proteins involved in cellular differentiation and stimulus response compared to normal and regurgitating/vomiting dogs ($p < 0.05$).

DISCUSSION

In this pilot study, untargeted LC-MS was used to evaluate the canine GF proteome and identify several candidate biomarker proteins demonstrating DA in health and disease in the dog. Proteins specific to the GI tract were identified in the OP of

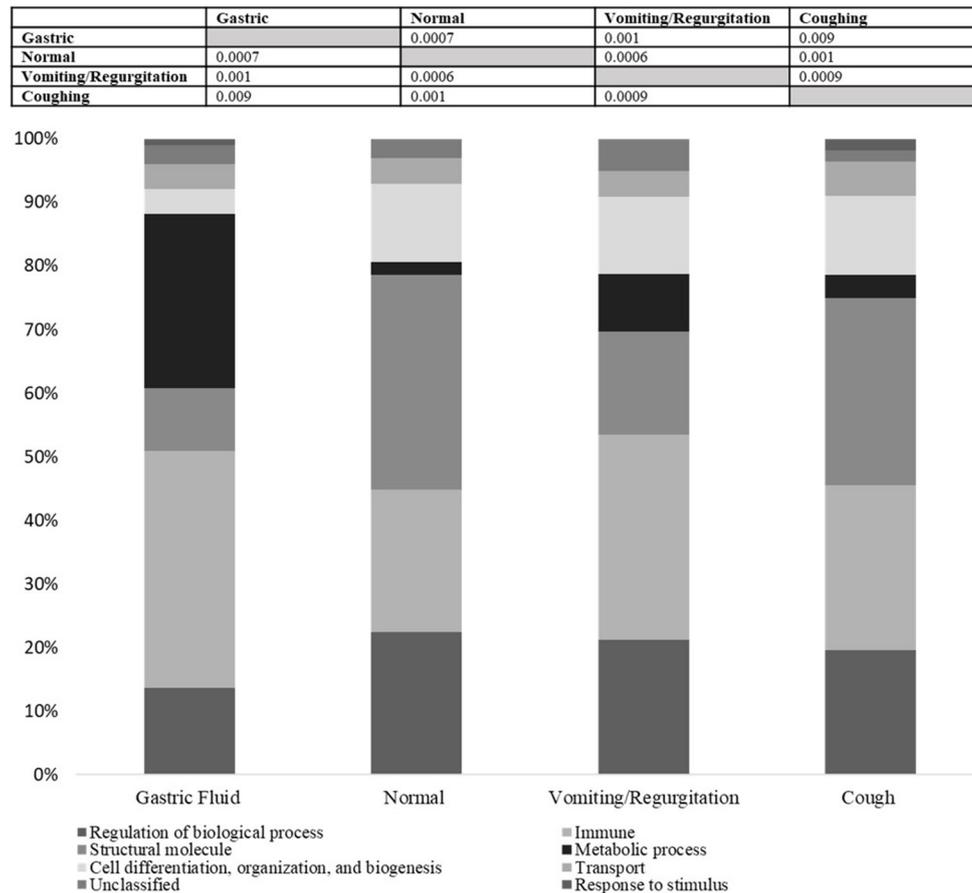


FIGURE 2 | All groups were statistically dissimilar by ANOSIM based on the biological function of identified proteins ($p \leq 0.001$). This suggests that the functional proteome differs by site (gastric fluid vs. oropharyngeal, OP) as well as between health and disease. The biological function profile for each group is displayed in the stacked bar chart above. Metabolic and immune proteins were over-represented in gastric fluid compared to normal dog OP swabs ($p < 0.05$). Metabolic proteins were over-represented in vomiting/regurgitating dog OP swabs compared to healthy dogs ($p < 0.05$) as were proteins with immunologic function ($p < 0.05$). Coughing dog OP swabs had increased the number of proteins involved in cellular differentiation and stimulus response compared to normal and regurgitating/vomiting dogs ($p < 0.05$).

vomiting/regurgitating and coughing dogs but not of healthy dogs. This suggests a potential role for reflux in the pathogenesis of respiratory disease in dogs and the possible utility of protein biomarkers as a screening tool for dogs with EERD and aspiration. Reflux and aspiration contribute significantly to the pathogenesis and progression of respiratory disease in people (6–9, 24). While a similar relationship is suspected in veterinary patients, a readily available diagnostic test capable of detecting EERD and aspiration is lacking (25), and the present proteomics approach shows promise. Though further studies are needed, significant between-dog variability may suggest an improved utility of biomarker panels over individual proteins.

Understanding the link between reflux and aspiration in clinical patients is critically important as people with a reflux-associated respiratory disease have demonstrated reductions in both disease exacerbation and declines in lung function following treatment targeting GERD and EERD (7). A similar targeted therapeutic approach may similarly be beneficial in dogs with

airway, interstitial, and/or pulmonary parenchymal diseases. In experimental canine models, a link between respiratory disease, reflux, and aspiration in dogs has been demonstrated, with bronchoconstriction, laryngospasm, laryngeal paresis/paralysis, and microaspiration occurring in response to the application of acid and digestive enzymes to the esophagus and larynx (26–29). In companion dogs, this association is less clear. In brachycephalic dogs, an animal model for obstructive sleep apnea in people, treatment for presumptive GERD/EERD significantly improved the clinical signs of brachycephalic airway syndrome and minimized post-surgical complications (24, 25, 30). As such, treatment for EERD may also benefit dogs with other naturally developing respiratory disorders in which reflux and repetitive microaspiration play a role in disease development, progression, and exacerbation (12). Furthermore, similarities in anatomy, physiology, and several pathologic disorders make experimental and naturally occurring canine models integral to translational research in humans (31–33).

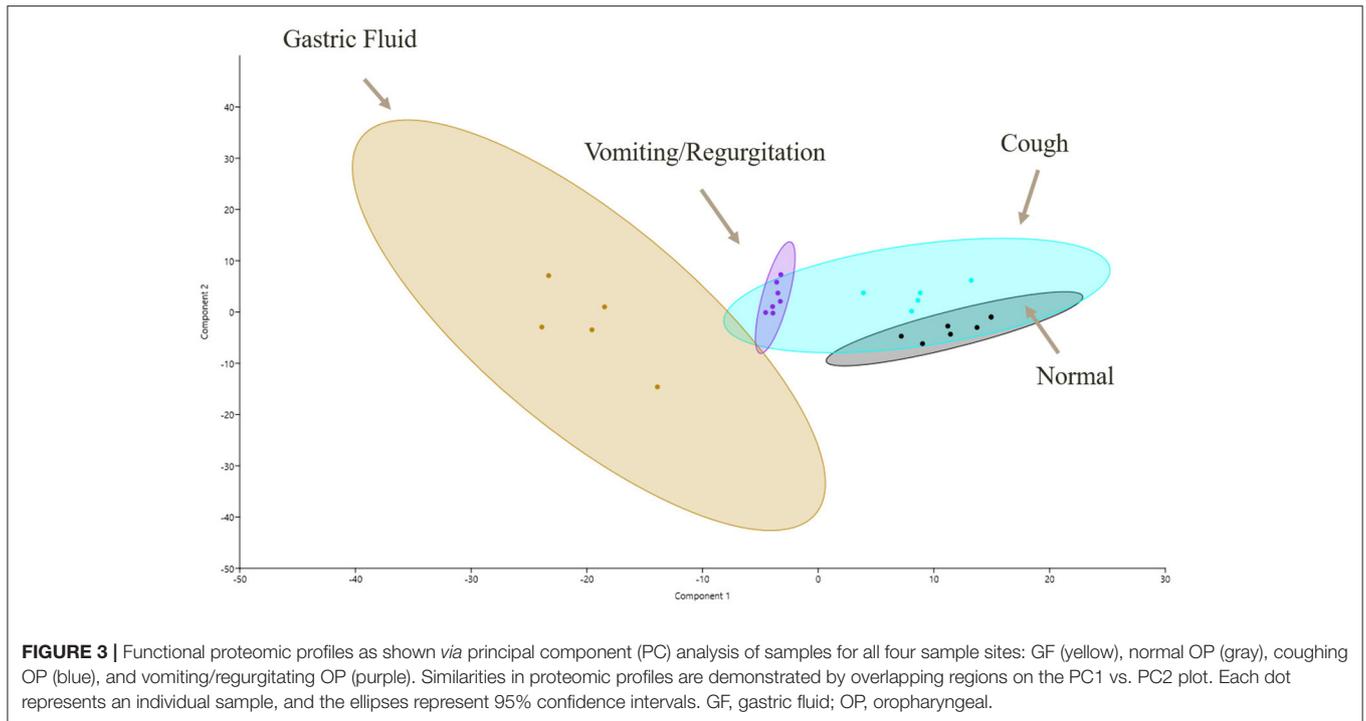


TABLE 2 | Metabolic proteins demonstrating increased abundance (GF vs. normal) and relative abundance in GF based on normalized spectral abundance factors are provided.

Metabolic protein	Tissue of origin	Relative abundance (gastric fluid)
Double-headed protease inhibitor	Salivary	1/230
Pancreatic lipase	Pancreatic	4/230
Chymotrypsin-C	Pancreatic	12/230
Pancreatic alpha-amylase	Pancreatic	16/230
Anionic trypsin	Pancreatic	19/230
Chymotrypsin	Pancreatic	37/230
Pancreatic secretory granule protein	Pancreatic	42/230
Chymotrypsin-like elastase	Pancreatic	46/230
Zymogen granule membrane-associated protein	Pancreatic	48/230
Zymogen membrane granule protein	Pancreatic	50/230
Pancreatic triacylglycerol lipase	Pancreatic	54/230
Gastric lipase	Gastric	58/230
Collipase	Pancreatic	63/230
Bile salt-activated lipase	Pancreatic	66/230
Chymosin	Intestinal	72/230
Pepsin A	Gastric	86/230

Only metabolic proteins found within the 100 most abundant proteins are displayed. GF, gastric fluid.

Identifying dogs with naturally occurring GERD and EERD poses a diagnostic challenge as reliable, inexpensive, and minimally invasive diagnostic tests are lacking. In people,

biomarkers have been effectively utilized as a part of a multimodal approach to diagnosing reflux and aspiration-associated respiratory syndromes (AARS) (8, 18, 20, 34). Though biomarkers have been utilized effectively for a number of diseases in dogs, investigation into the biomarkers of reflux and aspiration has been rarely performed (2, 5, 13, 22). In this study, LC-MS was successfully used to generate an initial proteomic profile of canine GF to identify potential biomarker candidates. The principal component analysis demonstrated a complete separation, with a CI of 95%, of the GF proteome and normal dog OP proteome, suggesting that these two sites significantly differ with respect to their protein composition. This is a key initial finding supporting that, in the absence of reflux, there are no gastric proteins in the healthy dog OP and sets the stage to identify biomarker gastric proteins in this location in disease states.

Statistically significant DA for multiple proteins was identified between all groups compared to normal dogs. This finding suggests that the canine proteome differs not only by the site of collection [GF vs. OP (normal)] but also in health and disease (vomiting/regurgitating or coughing dogs vs. normal). The principal component analysis demonstrated an overlap between GF, vomiting/regurgitating, and coughing proteomes. Furthermore, GI-specific proteins were identified in dogs with vomiting/regurgitation as well as dogs with cough. No such proteins were found in healthy dogs. Though further studies investigating larger sample sizes are needed, this supports the hypothesis that GF biomarkers may be identified in vomiting/regurgitating and coughing dogs.

Gastric pepsin (pepsin A) is a component of the human gastric refluxate which has been used as a biomarker to identify patients with EER and aspiration (16, 18, 20, 21). While pepsin A was

identified in canine GF, it was low in abundance. Furthermore, pepsin was not identified in OP swabs from any dog, including those with documented regurgitation/vomiting within 12 h of sample collection. It is possible that protein degradation, both *in vivo* and *ex vivo*, may have reduced the abundance of this protein below our limit of detection. The low abundance of pepsin A in GF and the lack of detection of pepsin A in OP swabs in vomiting/regurgitating dogs suggest this to be a less-than-ideal protein biomarker regardless of cause and represent a significant departure from the human literature (18, 34–38). Interestingly, pancreatic proteins were highly abundant in GF, likely suggesting that gastroduodenal reflux was a frequent occurrence in our population and supporting a biomarker study which identified bile in bronchoalveolar lavage fluid from dogs with pulmonary fibrosis (13). Salivary proteins were abundant in gastric fluid as well as in OP swabs in dogs with vomiting/regurgitation. While the amount of salivary proteins in gastric fluid may reflect the mode of collection (i.e., nasogastric or orogastric tube), it may also reflect the reflexive swallowing of salivary secretions (39). Increased abundance in dogs with vomiting/regurgitation may reflect bathing of the OP with concentrated salivary secretions from the gastric fluid, ptyalism secondary to nausea, or as a result of the esophagosalivary reflex which increases salivary secretions to protect against damage to the esophagus by gastric acid/digestive enzymes (40). A limitation of this study is the use of clinical dogs for the evaluation of gastric fluid. While evaluating healthy dogs would be ideal, the collection of gastric fluid could not be ethically justified in this population. Diet was not controlled in this population, other than all dogs received a commercial dog food. As such, the influence of diet on the GF proteome in dogs cannot be predicted. However, the fact that the documented differences in the proteome persist despite not controlling for diet may suggest that this is a true difference and not artifactual due to the influence of a common diet.

The presence of gastric proteins demonstrating an increased abundance in OP swabs from vomiting/regurgitating and coughing dogs compared to normal dogs opens doors for future biomarker validation studies in dogs with EERD and AARS. Important to future biomarker studies was the finding that the protein concentrations between dogs were extremely variable, echoing similar findings in people, thus suggesting limited utility for individual protein biomarkers and emphasizing the need for biomarker panels (41–43).

Biological protein function was determined for the 200 most abundant proteins per group, and a statistical evaluation by ANOSIM demonstrated that the OP swabs from normal, vomiting/regurgitating, and coughing dogs were statistically dissimilar, representing discrete functional proteomic profiles. This suggests that the differences in composition between groups may be enough to alter the functional resident proteome in

health and disease. In vomiting/regurgitating dogs, proteins with metabolic (i.e., enzymatic) functions were found in increased abundance compared to those in normal dogs. This may reflect a contribution from the GF proteome, though the overall functional proteomic profiles were still considered dissimilar between these two groups.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: massive.ucsd.edu (proteomxchange), identifier MSV000087178.

ETHICS STATEMENT

The animal study was reviewed and approved by University of Missouri IACUC #9154. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

MG, CR, and HR contributed to the writing of the manuscript. MG and HR were involved with sample preparation and data analysis. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fvets.2021.670007/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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