Comparison of complete blood count and biochemistry panels between cats with and without clinically apparent feline upper respiratory tract disease.

by
Sarah Duke

A THESIS

submitted to

Oregon State University

Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Biology
(Honors Associate)

Presented May 15, 2020
Commencement June 2020

AN ABSTRACT OF THE THESIS OF

Sarah Duke for the degree of Honors Baccalaureate of Science in Biology presented on May 15, 2020. Title: Comparison of complete blood count and biochemistry panels between cats with and without clinically apparent feline upper respiratory tract disease.

Abstract approved:_____

Brianna Beechler

Feline upper respiratory tract disease (FURTD) is a complex syndrome that affects domestic cats, with clinical signs including general malaise, ocular and nasal discharge, and oral pathology. Our study compared the presence or absence of five common FURTD pathogens and routine blood parameters between 18 participating cats. Owners tended to underreport FURTD signs, particularly oral pathology. Additionally, several cats that displayed clear FURTD signs tested negative for common FURTD pathogens, while two cats that did not have any owner-reported signs tested positive. Clinically ill cats had a suggestively higher neutrophil count ($n = 17$, $p = 0.0081$) and neutrophil-lymphocyte ratio ($n = 18$, $p = 0.048$) than clinically healthy cats, although the statistical significance was eliminated after correction for multiple comparisons. Cats that tested positive for at least one respiratory pathogen had a suggestively lower platelet count ($n = 13$, $p = 0.0615$) than cats that tested negative for all pathogens. Our findings suggested a weak association between neutrophilia and FURTD, which may potentially aid in early detection of the disease, even in patients with subclinical infections. However, further research with a larger study population may enable the acquisition of more statistically significant findings.

Key Words: feline, cat, FURTD, respiratory, blood, pathogen

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I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

_____

Sarah Duke, Author

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Introduction:

Feline upper respiratory tract disease is a common, multifactorial syndrome affecting cats and kittens, particularly those living in high-density environments such as animal shelters, catteries, or multi-cat households [1,2]. Clinical signs of FURTD include serous to mucopurulent ocular or nasal discharge, sneezing, epistaxis, and severe ocular, nasal, and/or oral pathology including inflammation and ulceration of mucous membranes and corneas. Signs can be accompanied by fever, anorexia, and general malaise [3,4]. Acute and chronic signs of FURTD are caused by the interaction of one or more pathogens, host susceptibility, and stress. Viral (feline herpesvirus 1 (FHV-1) or calicivirus (FCV)), secondary bacterial (e.g. *Pasteurella multocida*, *Staphylococcus* and *Streptococcus* spp., and various anaerobes), primary bacterial (e.g. *Bordetella bronchiseptica*, *Chlamydophila felis*, and *Mycoplasma* spp.) and, less commonly, fungal (e.g. *Cryptococcus neoformans*) pathogens have all been isolated from cats with signs of FURTD and are implicated in the disease process [1,2,4,5]. Besides the aforementioned pathogens, many other respiratory pathogens can contribute to the clinical presentations of the disease [5].

Common respiratory pathogens involved in the development of FURTD (FHV-1, FCV, *B. bronchiseptica*, and *C. felis*) are shed in respiratory mucosa (oral, nasal, and ocular), and are spread primarily through direct contact. Transmission via fomite is possible, particularly when pathogen loads are high [6]. Many of the viral pathogens are shed for long periods of time post-infection and persist in the environment for several weeks [7,8]. Transmission is further facilitated by the presence of asymptomatic carrier cats. For example, cats infected with FHV become lifelong carriers. The virus exists in a latent state in nervous tissue and is shed during times of stress, such as boarding, immunosuppressant treatment, travel or other illness [7]. Cats with FCV shed continuously after infection, but are less likely to become lifelong carriers and shedding decreases over time [8]. Although vaccines for both FHV and FCV exist and protect against disease, they do not prevent infection or the development of a carrier state [7,8]. Crowded environments such as multi-cat households, shelters and catteries promote pathogen spread. The number of cats in close confinement is a significant predictor of FURTD [9]. For example, households of 4–10 cats and catteries containing >50 cats have significant negative associations with respiratory tract disease [9]. In animal shelters within the United States, FURTD is a common cause for euthanasia. Dinnage et al. reported on one large urban shelter where FURTD affected one third of cats during their year-long study period, and more than 30% of affected kittens and over 60% of adult cats were euthanized due to FURTD [1]. The high rate of morbidity and mortality associated with FURTD in our domestic cat populations make increasing our understanding of this disease process a priority in feline medicine.

Diagnosis of FURTD is often challenging due to the variability of clinical signs and redundancy of signs with other disease states. For instance, atypical acute FHV infections may manifest as fever, depression, and anorexia with ulcerated nasal and facial lesions instead of the more commonly recognized manifestations of sneezing, nasal discharge, and conjunctivitis [7]. FCV is most commonly associated with oral ulcers , gingivitis, and stomatitis, which may also be associated with Bartonellosis (*Bartonella henselae*), toxicities, or uremia [8,10]. Subclinical infections are also common, as these pathogens tend to go into latent states and recrudesce in times of stress [6,7,8]. Quantitative real-time PCR (qPCR) is the preferred method of screening for respiratory pathogens [6,7,8,11]. Alternatively, rhinoscopy with nasal biopsy may provide a more definitive diagnosis, as false negative results can occur [11,12]. At this time, the relationship between clinicopathologic data and presence of FURTD-associated pathogens has not been explored. Any association between abnormalities in complete blood count (CBC), serum biochemistry and

FURTD may potentially aid in early detection of the disease, and in patients with subclinical infections.

The primary objective of the study was to compare clinicopathologic changes between three groups of subjects: clinically healthy vs. clinically ill, pathogen positive vs. pathogen negative, and clinically ill cats that were pathogen positive vs. clinically ill cats that were pathogen negative. For the purposes of this project, cats were classified as clinically ill if FURTD signs were present upon examination by a veterinarian at the time of the study, even if owners did not previously report signs. Cats were classified as clinically healthy if FURTD signs were not present upon examination by a veterinarian at the time of the study, and had no owner-reported history of FURTD signs. We expected to see statistically significant differences between blood parameters for each of the three comparisons, particularly parameters that change with inflammation (white blood cell counts).

Materials and Methods:

Eight households, and a total of 18 cats, were included in this study as part of research on feline upper respiratory microbiomes. According to selection criteria, each household included at least one cat with chronic clinical FURTD, and at least one cat which had never displayed FURTD signs. Study subjects were recruited in Corvallis, Oregon, USA using multiple outlets: an email request targeting the entire Oregon State University Corvallis campus community; an ad in the OSU Today campus newsletter; flyers posted across campus; an email request sent to local veterinary hospitals; and via conversations with study personnel. To be included in the study, cats needed to meet the following criteria: all cats must 1) be spayed or neutered, 2) have proof of a current rabies vaccination, 3) be at least 1 year old, 4) live with at least one other cat for a minimum of 1 year, and 5) have no comorbidities that would impact results. Prior to beginning a physical examination and sample collection, a full history was obtained by a veterinarian for each patient, including: age, breed, vaccine history (rabies, FVRCP, and FeLV), outdoor access, diet type (dry or canned) and brand, current medication(s), use of flea, tick and heartworm preventatives, most recent deworming, most recent veterinary visit and reason for the visit, and most recent FURTD signs, if any. FURTD signs were identified by owners through the cats' clinical history (Table 1) and by veterinarians during the physical exam (Table 2). For the purpose of this study, cats with at least one veterinarian-reported sign of FURTD were considered clinically ill, and cats without any veterinarian-reported FURTD signs were categorized as clinically healthy.

Table 1. Clinical signs of FURTD in domestic cats, reported by owners as part of clinical history prior to physical exam (Appendix A)[13,14]

Eyes	**Nose**	**Mouth**
Ocular discharge/crusts	Sneezing	Gingival inflammation
Conjunctivitis	Nasal discharge	Oral inflammation
Ocular swelling	Nasal crusts	Lingual inflammation
Ocular clouding	Nasal inflammation	Inflammation of fossa
Corneal ulceration		

Table 2. Clinical signs of FURTD detected by veterinarian during physical exam (Appendix B) [13,14,15].

Ocular	**Nasal & Respiratory**	**Oropharyngeal**

Bilateral serous to mucopurulent discharge	Bilateral serous to mucopurulent discharge	Stomatitis
Conjunctivitis	Sneezing	Glossitis
Chemosis	Rhinitis	Faucitis
Keratitis	Stertor	Gingivitis
Corneal ulceration		
Anterior uveitis		

Sample Collection and Processing:

Each participating cat was subjected to a thorough physical exam performed by an experienced clinical veterinarian (Beechler or Hanselmann) in their residences'. Immediately following the exam, four milliliters of blood were collected via peripheral venipuncture for serum biochemistry analysis, CBC (whole blood in EDTA), and feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), and heartworm antigen (*Dirofilaria immitis*) testing (serum) (Table 3, Table 4). Owners were asked to provide fresh fecal and urine samples from each participating cat within one week of sampling. Home urine samples were collected using nonabsorbent cat litter (Nosorb®, product number 0878408000850). Serological evaluations for FeLV, FIV and heartworm, and fecal examinations were performed to rule out co-infections that could confound results. If comorbidities that could impact results were found, the cat was eliminated from the final assessment. All diagnostics except for feline respiratory pathogen screening were carried out at the OSU Carlson College of Veterinary Medicine (CCVM). Oronasopharyngeal swabs were collected using sterile synthetic swabs with plastic handles (Copan®, FLOQSwabsTM, Brescia, Italy) [9]. To maximize yield of genetic material for respiratory pathogen identification, swabs from the upper respiratory tract were pooled and stored in 5 mL of sterile phosphate-buffered saline (PBS)[11,16,17].

Samples were stored at 4℃ until processing within 24 hours of collection. Complete blood counts and serum chemistry analyses (Small Animal Complete Chemistry Profile) were carried out at the Oregon Veterinary Diagnostic Laboratory at OSU CCVM, with the exception of subject 5, whose blood samples were collected independently of the study at a local veterinary hospital and analyzed by Idexx laboratories (Adult Annual with Urinalysis, 35949999). FeLV, FIV, and heartworm antigen tests were performed using the Idexx SNAP® Feline Triple Test (product number 99-0000673) in a shared laboratory facility at OSU CCVM. Respiratory swabs were stored at -80℃ until their submission to the Real-time PCR Research and Diagnostics Core Facility at the University of California, Davis School of Veterinary Medicine for primary FURTD pathogen testing (FHV-1, FCV, *C. felis*, *Mycoplasma* spp., and *B. bronchiseptica*) using quantitative real-time PCR [9].

Fecal samples were examined for intestinal parasites via sugar flotation at the OSU CCVM biomedical science laboratory. Briefly, a 3-gram sample of feces from each cat was mixed with 10 mL of Sheather's solution in a cup. The Sheather's solution was prepared by combining 227 grams of granulated sugar with 177.5 mL of tap water and heating until all sugar granules were dissolved [18]. The specific gravity of the solution was 1.27. The Sheather's solution-feces mixture was poured through a strainer into a 15 mL centrifuge tube, which was then centrifuged at 500 RCF for 3 minutes. Once completed, the tube was filled to the brim with Sheather's solution and a glass coverslip placed on the meniscus for 15 minutes. The coverslip was then lifted vertically off the tube, placed on a slide, and examined with a light microscope at 10X and 40X.

Table 3. Descriptions and implications of disease processes on serum biochemistry parameters measured in domestic cats [15, 19, 20]. "↑" = predicted increase with presence of FURTD signs and/or pathogens, "↓" = predicted decrease with presence of FURTD signs and/or pathogens, "-" = no predicted changes with presence of FURTD symptoms and/or pathogens.

Parameter	Unit of Measurement	Description	Predicted Effects of FURTD
Blood Urea Nitrogen (BUN)	mg/dL	Chemical waste product produced by hepatic protein metabolism and freely filtered by the kidneys. Abnormal results may occur with high or low protein diets, abnormal glomerular filtration rates, and hepatic dysfunction.	-
Glucose (Glu)	mg/dL	Energy source for cellular mechanisms. Elevations may be due to stress, diabetes mellitus and hyperadrenocorticism. Decreases may be attributed to insulinomas or acute febrile illness.	-
Creatinine (Crea)	mg/dL	Waste products produced by striated muscle and freely filtered by kidneys. Elevations are primarily associated with low glomerular flow rates (renal disease). Decreases may result from sarcopenia and high metabolic states associated with hyperthyroidism.	-
Cholesterol (Chol)	mg/dL	Major structural component of cell membranes. Used to make hormones, fat-soluble vitamins, and bile acids Changes may be due to endocrine disorders, biliary disease and high fat diets.	-
Total Protein (TP)	g/dL	Measure of albumin and globulins in blood. Increases are often associated with dehydration or chronic inflammation. Decreases may be caused by blood loss, protein-losing enteropathy/nephropathy or hepatic dysfunction.	↑
Albumin (Alb)	g/dL	Protein manufactured by the liver. Promotes fluid retention in the bloodstream. Decreases are usually associated with hepatic insufficiency or	-

		loss, and increases are seen with dehydration.	
Bilirubin (Bili)	mg/dL	Waste product of heme breakdown. Increases may occur with hepatic insufficiency and hemolysis.	-
Creatine Kinase (CK)	U/L	Cytosolic enzyme relatively specific to muscle tissue in serum. Increases are associated with muscle damage, low potassium, and hypoxemia.	-
Alkaline Phosphatase (ALP)	U/L	Membrane-associated enzyme produced in many tissues. Serum or plasma levels increase with liver disease and bone turnover in cats.	-
Gamma-Glutamyl Transferase (GGT)	U/L	Membrane-associated enzyme. Increases in serum/plasma are typically associated with biliary disease.	-
Alanine Aminotransferase (ALT)	U/L	Cytosolic enzyme produced mainly by hepatocytes. Increases in serum levels are often associated with liver damage or inflammation.	-
Sodium (Na^+)	mEq/L	Electrolyte vital to proper muscle and nerve function, and maintenance of normal blood pressure and fluid balance. Increases may be associated with dehydration and renal failure, among other maladies. Decreases are usually due to loss.	-
Potassium (K^+)	mEq/L	Electrolyte vital to regulation of fluid balance, muscle and nerve function. Increases may occur with decreased renal excretion and acidosis. Decreases are associated with anorexia, increased loss (vomiting, elevated tubular flow rate) and alkalosis.	-
Chloride (Cl^-)	mEq/L	Electrolyte that aids in regulation of fluid balance and blood pH. Loss is often due to increased excretion or vomiting. Independent increase is usually compensatory due to loss of bicarbonate.	-
Calcium (Ca^{2+})	mEq/L	Most common mineral in the body, necessary for muscle contraction, bone	-

		and tooth formation, cardiac function, and blood clotting. Decreases are common with loss of albumin, intestinal or renal disease and vitamin D deficiency.	
Phosphorus	mEq/L	Element vital to bone growth, energy storage, and nerve and muscle production. Decreases are primarily due to lack of intake or vitamin D deficiency. Increases are common with disorders of glomerular filtration including renal dysfunction in cats.	-
Total Carbon Dioxide (TCO_2)	mEq/L	Chemical substance that acts a buffer for blood pH, as most TCO_2 is comprised of bicarbonate. Changes result from electrolyte and fluid imbalances and hypoxemia. Increases are usually attributed to vomiting. Decreases occur with renal disease, diarrhea, poor perfusion and starvation.	-
Anion Gap (AG)	mEq/L	A measurement of the interval between the sum of routinely measured cations (sodium and potassium) minus the sum of the routinely measured anions (chloride and bicarbonate) in the blood. Increases are usually due to presence of organic acids such as lactate, ketones or uremic toxins. Decreased AG is usually due to loss of albumin.	-

Table 4. Descriptions and implications of FURTD disease processes on complete blood count parameters measured in domestic cats [15,19,20]. "↑" = predicted increase with presence of FURTD signs and/or pathogens, "↓" = predicted decrease with presence of FURTD signs and/or pathogens, "-" = no predicted effect with presence of FURTD symptoms and/or pathogens.

Parameter	**Unit of Measurement**	**Description**	**Predicted Effects of FURTD**
White Blood Cell Count (WBC)	cells/µL	Number of white blood cells per microliter of blood. Changes are associated with a wide variety of causes. Inflammation and stress may increase counts, while decreases may be associated with bone marrow disease.	↑
Red Blood Cell	cells/10^6 µL	Number of red blood cells per unit of blood.	↓

Count (RBC)		Decreases (anemia) may be attributed to hemolysis, hemorrhage, or chronic disease (including inflammation, infection, bone marrow disease, and endocrine diseases). Increases may be due to dehydration.	
Hemoglobin (Hg)	g/dL	Protein in RBCs responsible for transporting oxygen in the blood. Increases are due to dehydration. Hemorrhage, hemolysis, and bone marrow disease may cause increases or decreases.	-
Hematocrit (HCT)	%	The ratio of the volume of red blood cells to the total volume of blood. May increase due to dehydration. Decreases are associated with hemorrhage, hemolysis, and bone marrow disease.	-
Packed Cell Volume (PCV)	%	The volume of packed red blood cells divided by the total volume of the blood sample after centrifugation. Similar to HCT, but not affected by agglutination.	-
Mean Cell/Corpuscular Volume (MCV)	fL	Measure of the average volume of a red blood cell corpuscle. May decrease with nutritional (iron) deficiencies. Increases are often due to the presence of young RBCs in regenerative anemia.	-
Mean Corpuscular Hemoglobin (MCH)	pg	Calculated value that represents the amount of hemoglobin per red blood cell. Usually changes with MCHC.	-
Mean Corpuscular Hemoglobin Concentration (MCHC)	g/dL	Calculated value that represents the percent of a red blood cell that contains hemoglobin (hemoglobin divided by PCV).	-
Reticulocytes	% and cells/µL	Immature, anucleated RBCs that are used to determine whether or not anemia is regenerative. Decreases are found in cases of non-regenerative anemia or bone marrow defects, and increases occur in regenerative anemia or lead toxicity.	-
Mean Platelet Volume (MPV)	fL	Measure of the mean volume of platelets. Useful in distinguishing between causes of thrombocytopenia.	-
Red Blood Cell Distribution Width (RCDW)	%	A calculated index of the variation in cell volume within the red blood cell population. May be decreased by high numbers of small RBCs (e.g. iron deficiency anemia) or increased by high numbers of large, immature RBCs (e.g. regenerative anemia).	-

Platelet Count	x1000/µL	Manual or calculated estimation of the number of platelets per volume of blood. May increase with autoimmune disorders and trauma. Decreases are frequently artifact (due to platelet clumping), but may also be attributed to viral infections [20]. Chronic inflammation may cause both increases and decreases in platelet counts [16,20].	↑ or ↓
Plasma Protein (PP)	g/dL	Proteins present in whole blood that perform multiple essential functions including oncotic pressure, lipid, hormone and vitamin transport, hemostasis and immune system maintenance. Includes albumin, globulins and coagulation proteins (the latter are not present in serum). Increases are often associated with dehydration or chronic inflammation. Decreases may be caused by blood loss, protein-losing enteropathy/nephropathy or hepatic dysfunction.	↑
Neutrophils	cells/µL	Primary circulating leukocyte in the cat. Phagocytic WBC that circulates widely throughout the body, and responds quickly to pathogens. Changes result from stress, exogenous corticosteroids, and inflammation due to infectious and immune-mediated disease. Bacterial infection usually results in an increased WBC count, while viral infections result in a decrease.	↑ or ↓
Lymphocytes	cells/µL	WBCs involved in adaptive and innate immunity. Alterations can be physiologic (age and stress) or due to pathology including infectious disease (usually viral) or uncommonly in cats, lymphoid neoplasia. Increases are seen in cats under 6 months of age, and with chronic inflammatory disease and bacterial infection. Decreases are associated with viral infections.	↑ or ↓
Monocytes	cells/µL	Largest WBC involved in both innate and adaptive immunity. Elevations are due to more chronic inflammation. Monocytes become phagocytic macrophages when they leave circulation.	↑
Eosinophils	cells/µL	Type of WBC which participate in allergic reactions and modulate inflammatory responses. Changes are usually due to inflammation associated with parasites and allergic reactions.	-
Basophils	cells/µL	Type of WBC which mediates allergic reactions. Changes are usually due to allergic reactions or parasites.	-

Neutrophil-Lymphocyte Ratio	N/A	Calculated value used as an indicator of systemic inflammation [20]. Elevated ratios indicate inflammation with a higher susceptibility to viral infection.	↑

Statistical Analyses:
Blood parameters were compared between clinically ill and clinically healthy groups, between pathogen-positive and pathogen-negative groups, and between clinically ill cats who tested positive for at least one FURTD pathogen and clinically ill cats who tested negative for all FURTD pathogens. Because of a small sample size and data that was not normally distributed, the Mann-Whitney test (also called the Wilcox test) was utilized to compare medians between groups. P-values were adjusted using the Holm-Bonferroni method to account for multiple comparisons. For all statistical analyses, significance was defined as $P < 0.05$. All available blood parameters from the CBC and chemistry panels were compared.

Results:
Study population—
Eighteen total cats from eight different households were recruited for this study. Seven households contributed 2 cats each, and one household contributed 4 cats. All participating cats were neutered, and fell into either adult, mature, or senior age categories (Table 5). No geriatric (>15 years), junior cats (7 months to 2 years), or kittens (birth to 7 months) were included. Of the eighteen total cats, nine (50%) were overweight and seven (39%) were obese (Table 5). The majority of participating cats (13 of 18) were domestic breeds (shorthair, medium hair, or long hair), four were mixed breeds, and one was Siamese (Table 5).

Table 5. Description of study population. Number of cats in defined age groups, sexes, breeds, weights, and body condition scores (1 = emaciated, 9 = morbidly obese).

Age (Years)	Number of Cats	Sex	Number of Cats
Adult (3 - 6 years)	9	Male, Castrated	11
Mature (7 - 10 years)	6	Female, Spayed	7
Senior (11 - 14 years)	3		
Breed		**Body Weight (lb)**	
Domestic Shorthair	11	7 - 10	5
Domestic Medium Hair	1	10.1 - 13	5
Domestic Longhair	1	> 13.1	8
Siamese	1	**Body Condition Score (1-9)**	

Siamese Mix	2	Ideal (4-5)	2
Manx/Desert Lynx	2	Overweight (6-7)	9
		Obese (8-9)	7

Physical Examination and FURTD Signs—
Of eighteen total subjects, nine had a history of owner-reported FURTD signs, and the other nine did not. However, eleven cats had at least one FURTD sign detected by a veterinarian during physical examination, while the remaining seven were deemed clinically healthy in the context of respiratory disease (Table 6). The most commonly recorded FURTD-associated signs during physical exam were sneezing and ocular discharge/crusts, followed by sneezing and nasal discharge/crusts (Figure 1). All eleven clinically ill cats presented with at least two co-occurring signs (Figure 2). Overall, participating cats had an average of 2.9 co-occurring signs. One subject had six co-occurring FURTD signs; more than any other subject. Two subjects in this study had clinical exam findings consistent with upper respiratory disease, while owners did not report signs. One cat did not have any owner-reported signs, but presented with ocular discharge, and gingivitis during the physical exam. This cat also had stertor, which was associated with stenotic nares, and therefore not a symptom of FURTD. Similarly, one subject did not have any owner-reported signs, but presented with gingivitis and faucitis during physical examination. All other ill participants had signs that were both owner-reported and present upon physical examination.

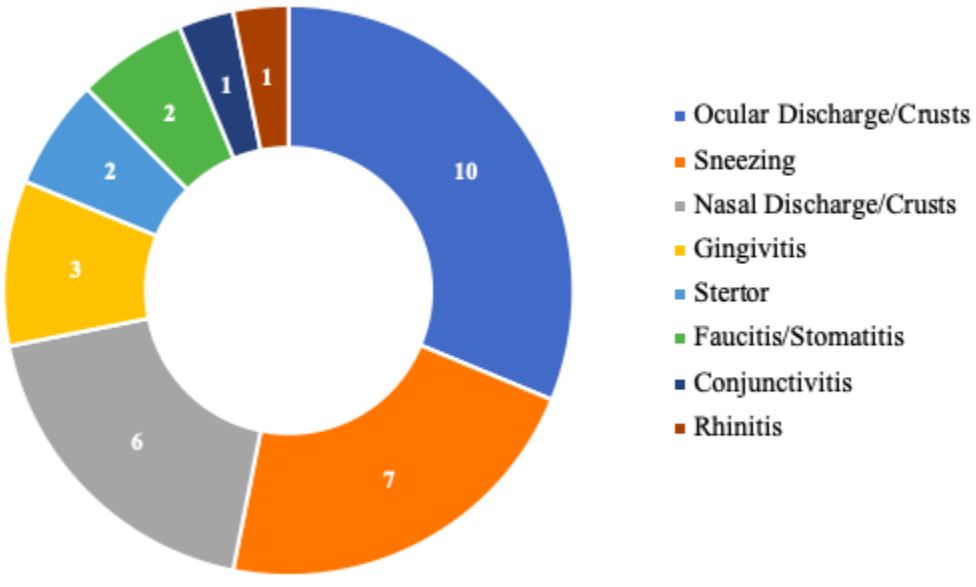

Figure 1. Number of cats who presented with FURTD signs during physical exam.

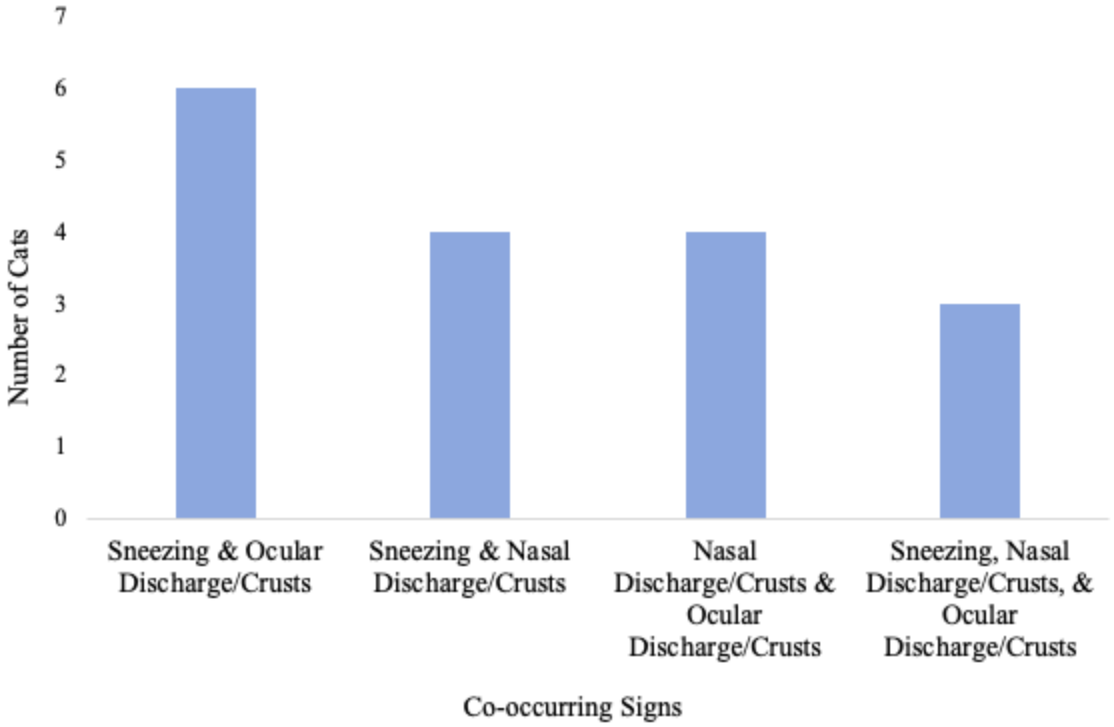

Figure 2. Most common co-occurring FURTD signs.

Feline respiratory pathogen qPCR —
Swabs from all 18 cats were tested for feline respiratory pathogens. Of the eleven cats that showed clinical signs of FURTD, four cats from two households tested positive for at least one respiratory pathogen. All qPCR positive cats were infected with FHV-1. Two cats were co-infected with FHV-1 and FCV. A third cat tested positive for FHV-1, *Bordetella bronchiseptica* and *Mycoplasma felis*. Three of the four test-positive cats lived in the same household (Table 6). No cats tested positive for *Chlamydophila felis/psittaci*, and none of the clinically healthy subjects tested positive for any respiratory pathogens. For one cat, the sample failed quality control for FCV; thus, results for FCV are not available for this individual.

Table 6. Quantitative real-time PCR feline respiratory panel results and presence or absence of FURTD signs as determined by owners (historically) and veterinarians (during study examination).

Owner-Reported signs	**Number of Cats**
Yes	9
No	9
Veterinarian-Reported signs	
Yes	11

No	7

Pathogen Test Result	
Positive	4
Negative	14

Table 7. Quantitative real-time PCR results and concurrent clinical signs of FURTD for both clinically ill and healthy cats, as determined by a veterinarian. (FHV-1 = Feline Herpesvirus 1, FCV = Feline Calicivirus, Bb = *Bordetella bronchiseptica*, Mf = *Mycoplasma felis*. "-" = qPCR-negative for all pathogens. Cats in the same household were assigned the same letter.)

House hold	Owner-reported FURTD status	Observed clinical signs	qPCR results
A	+	Ocular discharge, ocular crusts, sneezing, nasal discharge, rhinitis	-
A	-	None	-
B	+	Sneezing, nasal discharge	FHV-1
B	-	None	-
C	-	None	-
C	+	Ocular discharge	-
D	+	Ocular discharge, sneezing, nasal discharge, stertor, faucitis	-
D	+	Sneezing, nasal discharge, nasal crusts, ocular discharge, conjunctivitis, stomatitis	FHV-1, FCV
D	-	Ocular discharge, stenotic nares, stertor, gingivitis	FHV-1, Bb, Mf
D	-	Ocular discharge, ocular crusts, gingivitis, faucitis	FHV-1, FCV
E	+	Ocular discharge, sneezing, nasal discharge, stertor, gingivitis	-
E	-	None	-
F	+	Ocular crusts, sneezing	-
F	-	None	-
G	-	None	-
G	+	Ocular discharge, ocular crusts, sneezing, nasal discharge	-
H	+	Ocular crusts, sneezing	-
H	-	None	-

Blood Parameter Comparisons—

Complete blood count and chemistry panel parameters were compared between clinically ill (symptomatic) and healthy cats, between pathogen positive and pathogen negative cats, and between ill cats that tested positive for respiratory pathogens and ill cats that tested negative for respiratory pathogens. Complete blood counts were available for 9 of 11 ill subjects, and 4 of 7 healthy subjects. Complete blood count results were available for all 4 cats that tested positive for FURTD pathogens. Serum chemistry panels were available for all 18 subjects. All participating cats had platelet counts below the reference range provided by the Oregon Veterinary Diagnostic Lab (OVDL). This is likely attributed to clumping, which occurred in almost all whole blood samples. All participating cats also had Gamma-Glutamyl Transferase (GGT) below reference ranges (0 U/L, RR= 1-8 U/L), which is likely artifact.

Clinically Ill vs. Clinically Healthy:

Clinically ill cats appeared to have a higher absolute neutrophil count ($n = 13$, $p = 0.0081$), neutrophil percentage ($n = 13$, $p = 0.088$), and neutrophil-lymphocyte ratio ($n = 13$, $p = 0.034$) in comparison to clinically healthy cats (Table 8). Additionally, ill cats had a lower median lymphocyte percentage ($n = 13$, $p = 0.073$) and a slightly lower absolute lymphocyte count ($n = 13$, $p = 0.711$) in comparison to healthy cats (Table 8). No other CBC parameters appeared to differ between ill and healthy cats. Median plasma protein levels were above normal reference ranges provided by the OVDL for both ill and healthy cats, which could be associated with dehydration or artifact. Healthy cats had a median monocyte percentage that was slightly above reference range, but this value did not differ significantly from the median monocyte percentage of ill cats ($n = 13$, $p = 0.276$). Due to a small sample size, no significant differences at alpha = 0.05 remained for CBC parameters after correction for multiple comparisons.

Ill cats had slightly higher BUN ($n = 18$, $p = 0.0757$), serum albumin ($n = 18$, $p = 0.00357$), ALT ($n = 18$, $p = 0.0142$), and sodium ($n = 18$, $p = 0.0798$) levels compared to healthy cats (Table 9). No other chemistry parameters differed between ill and healthy cats. After correction for multiple comparisons, none of the aforementioned differences remained statistically significant at alpha = 0.05. However, serum albumin differences after correction ($n = 18$, $p = 0.064$) are significant at alpha = 0.10.

Table 8. Comparison of CBC parameters between clinically ill and healthy cats. "↑" = above upper limit of reference range, "↓" = below lower limit of reference range. Bolded parameters were predicted to change with FURTD status.

		Ill Subjects (n=9)			Healthy Subjects (n=4)				
Parameter	Unit of Measurement	Median	Q1	Q3	Median	Q1	Q3	p-value	Corrected p-value
WBC, total	**cells/µL**	**10500**	**9620**	**12730**	**7770**	**7130**	**8807.5**	**0.106**	**0.425**
RBC	**cells x 10_6/ µL**	**9.03**	**7.96**	**9.11**	**8.87**	**8.725**	**9.385**	**1**	**1**
Hemoglobin	g/dL	13.3	12.6	13.6	13.35	12.6	14.75	0.536	0.868
Hematocrit	%	41.95	37.45	43.675	43	39.15	46.425	0.825	0.943
PCV	%	40	36	41	40	38.5	43.5	0.574	0.868
MCV	fl	47.4	47	48	46.7	44.475	48.3	0.537	0.868
MCH	pg	14.7	14.1	15.3	15.15	14.275	16	0.394	0.868
MCHC	g/dL	30.6	30.2	31.2	32.7	30.7	35	0.246	0.8282
Red Cell Distribution Width	%	15.9	15.2	16.4	16.5	15.65	16.75	0.458	0.868
% Reticulocytes	%	0.5	0.4	0.5	0.55	0.425	0.6	0.762	0.943
# Reticulocytes	cells/µL	43300	40100	48300	52450 (↑)	43800	52500 (↑)	0.699	0.897
MPV	fl	19.4	17.3	22.7	20.8	19.4	21.8	0.578	0.868
Platelet Count	**x 1000 /µL**	**71 (↓)**	**39 (↓)**	**229 (↓)**	**102.5 (↓)**	**90.5 (↓)**	**132.25 (↓)**	**0.504**	**0.868**
Plasma Protein	**g/dL**	**8.6 (↑)**	**8 (↑)**	**9.2 (↑)**	**8.6 (↑)**	**8.05 (↑)**	**8.6 (↑)**	**0.453**	**0.868**
% Neutrophils	**%**	**71**	**69**	**81 (↑)**	**50.5**	**48.25**	**56.25**	**0.063**	**0.378**
% Lymphocytes	**%**	**19 (↓)**	**13 (↓)**	**20**	**31.5**	**21.975**	**41**	**0.052**	**0.378**
% Monocytes	**%**	**3**	**2**	**4**	**4.15 (↑)**	**3.225**	**5.25 (↑)**	**0.276**	**0.828**
% Eosinophils	%	4	3	5	5.2	4.75	9.05	0.101	0.425
% Basophils	%	0	0	0	0.5	0	1.25 (↑)	0.637	0.897
% Bands	%	0	0	0	0	0	0	N/A	N/A
% Metamyelocytes	%	0	0	0	0	0	0	N/A	N/A
% Myelocytes	%	0	0	0	0	0	0	N/A	N/A
% Other	%	0	0	0	0	0	0	N/A	N/A
Nucleated RBC	#/100 WBC	0	0	0	0	0	0	N/A	N/A
# Neutrophils	**cells/µL**	**7215**	**5595**	**9030**	**4374.5**	**4097.25**	**4586.25**	**0.0056**	**0.134**
# Lymphocytes	**cells/µL**	**1896**	**1497 (↓)**	**2816**	**2329**	**1654**	**3371.25**	**0.711**	**0.897**
# Monocytes	**cells/µL**	**347**	**236**	**399**	**356**	**218.75**	**502**	**0.825**	**0.943**
# Eosinophils	cells/µL	385	308	552	413	220.5	809 (↑)	1	1
# Basophils	cells/µL	0	0	136.5	41	0	98.25	1	1

# Bands	cells/µL	0	0	0	0	0	98.25	1	1
# Metamyelocytes	cells/µL	0	0	0	0	0	0	N/A	N/A
# Myelocytes	cells/µL	0	0	0	0	0	0	N/A	N/A
# Other	cells/µL	0	0	0	0	0	0	N/A	N/A
Neutrophil-Lymphocyte Ratio	**N/A**	**3.737**	**3.35**	**5.767**	**1.851**	**1.18**	**2.63**	**0.034**	**0.378**

Table 9. Comparison of serum chemistry parameters between clinically ill and healthy cats. "↑" = above upper limit of reference range, "↓" = below lower limit of reference range. Bolded parameters were predicted to change with FURTD status.

		Ill Subjects (n=11)			Healthy Subjects (n=7)				
Parameter	Unit of Measurement	Median	Q1	Q3	Median	Q1	Q3	p-value	Corrected p-value
BUN	mg/dL	26	25	27	24	22.5	24.5	0.0757	0.287
Creatinine	mg/dL	1.2	1	1.4	1.2	1	1.55	0.716	0.945
Glucose	mg/dL	80	69 (↓)	102	85	70.5	93	0.928	0.982
Cholesterol	mg/dL	171	137	256.5 (↑)	172	137	204.5 (↑)	0.860	0.968
Total Protein	**g/dL**	**7.6**	**7.15**	**7.85**	**7.7**	**7.2**	**8.6 (↑)**	**0.555**	**0.945**
Albumin	g/dL	3.5	3.45	3.75	3.3	3	3.3	0.00357	0.0643
Bilirubin, Total	mg/dL	0.2	0.15	0.2	0.1	0.1	0.25	0.735	0.945
CK	U/L	153	144	254	237	175.5	312.5 (↑)	0.126	0.378
Alkaline Phosphatase	U/L	17	12	20	18	17	21	0.412	0.927
GGT	U/L	0 (↓)	0 (↓)	0 (↓)	0 (↓)	0 (↓)	0 (↓)	N/A	N/A
ALT (SGPT)	U/L	56	48.5	71 (↑)	43	35	47	0.0142	0.128
Sodium	mEq/L	153	151.5	153.5	151	150	151.5	0.0798	0.287
Potassium	mEq/L	5	4.85	5.1	4.9	4.8	5.3	0.855	0.968
Chloride	mEq/L	114 (↓)	114 (↓)	116.5 (↓)	116 (↓)	114.5 (↓)	118.5	0.614	0.945
Calcium	mg/dL	9.5	9.05	10.2	9.3	8.95	9.6	0.389	0.927
Phosphorus	mg/dL	5.25	4.825	5.675	5	4.7	5.15	0.650	0.945
tCO2	mEq/L	18.8	16.3 (↓)	20.3	18.7	18.3	20.2	0.717	0.945
Anion Gap	mEq/L	24	22	26 (↑)	21	19.5	22	0.0437	0.262

Pathogen Positive vs. Pathogen Negative:

Cats that tested positive for at least one respiratory pathogen had a slightly higher absolute neutrophil count ($n = 13$, $p = 0.0755$) and plasma protein level ($n = 13$, $p = 0.0705$) compared to cats that tested negative for common respiratory pathogens (Table 10). Pathogen positive cats also had a lower platelet count ($n = 13$, $p = 0.0028$) compared to pathogen negative cats (Table 10). However, as previously stated, all cats tested had platelet counts that were artificially lowered due to clumping within samples. Thus, this difference between groups is unlikely to be caused by pathogen presence. Median plasma protein levels were above normal reference ranges provided by the OVDL for both pathogen positive and pathogen negative cats, which could be associated with dehydration or artifact. Although lymphocyte percentage did not differ significantly between pathogen positive and pathogen negative cats, the median lymphocyte percentage for pathogen positive cats was below reference ranges provided by OVDL, while median lymphocyte percentage for pathogen negative cats was within reference ranges. Although not statistically significant, pathogen positive cats had a slightly elevated NLR ($n = 13$, $p = 0.148$) in comparison to pathogen negative cats. This may have interesting implications for the role of pathogen presence on inflammatory response. No other CBC parameters differed between pathogen positive and pathogen negative cats. Due to a small sample size, no significant differences at alpha = 0.05 remained for CBC parameters after correction for multiple comparisons. However, median platelet counts significantly differed between groups after correction at alpha = 0.1.

Pathogen positive cats had lower BUN ($n = 18$, $p = 0.0249$) and ALT ($n = 18$, $p = 0.0143$) compared to pathogen negative cats (Table 11). Median serum albumin levels were slightly lower in pathogen positive cats than in pathogen negative cats ($n = 18$, $p = 0.0927$) (Table 11). Pathogen positive cats also had a higher total protein content ($n = 18$, $p = 0.0431$) than pathogen negative cats (Table 11). Interestingly, the median total protein value for the pathogen positive group was above reference range, while the median total protein value for the pathogen negative group was within reference range. None of the serum chemistry differences remained statistically significant at alpha = 0.05 after correction for multiple comparisons.

Table 10. Comparison of CBC parameters between respiratory pathogen positive and pathogen negative cats. "↑" = above upper limit of reference range, "↓" = below lower limit of reference range. Bolded parameters were predicted to change with FURTD status.

		Pathogen Positive Subjects (n=4)			Pathogen Negative Subjects (n=9)				
Parameter	Unit of Measurement	Median	Q1	Q3	Median	Q1	Q3	p-value	Corrected p-value
WBC, total	**cells/µL**	**10240**	**9890**	**12210**	**8200**	**7710**	**10920**	**0.504**	**0.852**
RBC	**cells x 10_6/µL**	**8.495**	**7.71**	**9.05**	**9.01**	**8.77**	**9.4**	**0.414**	**0.759**
Hemoglobin	g/dL	12.65	11.8	12.875	13.6	12.7	14	0.164	0.450
Hematocrit	%	39.45	36.825	41.325	44.5	40.5	45.5 (↑)	0.148	0.450
PCV	%	36.5	34.75	38	40.5	39.25	42	0.197	0.450
MCV	fl	47.2	46.525	47.55	47.1	46.3	49.4	0.817	1
MCH	pg	14.4	13.95	14.975	15.2	14.3	15.5	0.394	0.759
MCHC	g/dL	31.1	30.425	31.65	30.6	30.4	32.4	0.877	1
Red Cell Distribution Width	%	15.7	15.375	16.1	16.15	15.1	16.425	1	1
% Reticulocytes	%	0.45	0.375	0.55	0.5	0.5	0.6	0.862	1
# Reticulocytes	cells/µL	44250	36075	49525	44200	40100	52500 (↑)	0.938	1
MPV	fl	20.2	17.5	23.4	20.05	17.825	21.3	1	1
Platelet Count	**x 1000 /uL**	**36 (↓)**	**31.25 (↓)**	**43.25 (↓)**	**109 (↓)**	**89 (↓)**	**229 (↓)**	**0.00280**	**0.0615**
Plasma Protein	**g/dL**	**9.1 (↑)**	**8.9 (↑)**	**9.325 (↑)**	**8.05 (↑)**	**8 (↑)**	**8.6 (↑)**	**0.0705**	**0.450**
% Neutrophils	**%**	**73**	**70.5**	**77.5 (↑)**	**67**	**49**	**71**	**0.163**	**0.450**
% Lymphocytes	**%**	**16 (↓)**	**12 (↓)**	**19.75 (↓)**	**21**	**19 (↓)**	**40**	**0.141**	**0.450**
% Monocytes	**%**	**3**	**2**	**5 (↑)**	**3**	**3**	**5 (↑)**	**0.938**	**1**
% Eosinophils	%	4.5	3.5	5	4	3	5.4	0.815	1
% Basophils	%	0	0	0.75	0	0	1	1	1
% Bands	%	0	0	0	0	0	0	N/A	N/A
% Metamyelocytes	%	0	0	0	0	0	0	N/A	N/A
% Myelocytes	%	0	0	0	0	0	0	N/A	N/A
% Other	%	0	0	0	0	0	0	N/A	N/A
Nucleated RBC	#/100 WBC	0	0	0	0	0	0	N/A	N/A
# Neutrophils	**cells/µL**	**8122.5**	**7132.75**	**9850.25**	**4890**	**4485**	**5595**	**0.0755**	**0.450**
# Lymphocytes	**cells/µL**	**1573.5**	**1174.5 (↓)**	**2375.75**	**2075**	**1542**	**2936**	**0.330**	**0.726**
# Monocytes	**cells/µL**	**373**	**312.75**	**491.75**	**282**	**220**	**463**	**0.711**	**1**
# Eosinophils	cells/µL	442	341.25	591	382	294	552	0.817	1

# Basophils	cells/µL	105	52.5	202	0	0	82	0.303	0.726
# Bands	cells/µL	0	0	0	0	0	0	N/A	N/A
# Metamyelocytes	cells/µL	0	0	0	0	0	0	N/A	N/A
# Myelocytes	cells/µL	0	0	0	0	0	0	N/A	N/A
# Other	cells/µL	0	0	0	0	0	0	N/A	N/A
Neutrophil-Lymphocyte Ratio	**N/A**	**4.6996**	**3.53**	**6.714**	**3.0931**	**1.225**	**3.737**	**0.148**	**0.450**

Table 11. Comparison of serum chemistry parameters between respiratory pathogen positive and pathogen negative cats. "↑" = above upper limit of reference range, "↓" = below lower limit of reference range. Bolded parameters were predicted to change with FURTD status.

		Pathogen Positive Subjects (n=4)			Pathogen Negative Subjects (n=14)				
Parameter	Unit of Measurement	Median	Q1	Q3	Median	Q1	Q3	p-value	Corrected p-value
BUN	mg/dL	21.5	19.75	23.25	25	24	26.75	0.0249	0.224
Creatinine	mg/dL	1.25	1.15	1.325	1.15	1	1.675	0.873	0.988
Glucose	mg/dL	93	85	98.5	76.5	68.75	85.75	0.366	0.859
Cholesterol	mg/dL	158.5	122.25	230.25 (↑)	171.5	148.5	212.75 (↑)	0.878	0.988
Total Protein	**g/dL**	**8.4 (↑)**	**7.775**	**9.025 (↑)**	**7.35**	**6.975**	**7.925**	**0.0431**	**0.258**
Albumin	g/dL	3.15	2.825	3.3	3.35	3.3	3.575	0.0927	0.417
Bilirubin, Total	mg/dL	0.1	0.1	0.15	0.2	0.1	0.2	0.335	0.859
CK	U/L	236.5	176.5	344 (↑)	175.5	153.25	317.75 (↑)	0.382	0.859
Alkaline Phosphatase	U/L	18	17.5	19.5	18	13	21.5	0.831	0.988
GGT	U/L	0 (↓)	0 (↓)	0 (↓)	0 (↓)	0 (↓)	0 (↓)	N/A	N/A
ALT (SGPT)	U/L	34.5	31.5	37.75	47.5	44	60.5	0.0143	0.224
Sodium	mEq/L	151.5	150.75	152	151	150	153	0.957	1
Potassium	mEq/L	5.1	4.85	5.875 (↑)	4.95	4.825	5.1	0.556	0.988
Chloride	mEq/L	115 (↓)	113.75 (↓)	116.75 (↓)	115 (↓)	114 (↓)	118	0.667	0.988
Calcium	mg/dL	9.45	8.15	9.6	9.25	8.925	10.075	0.832	0.988
Phosphorus	mg/dL	4.85	4.65	5.075	5	4.8	5.7	0.495	0.988
tCO2	mEq/L	19.8	18.7	21.1	18.6	16.925 (↓)	19.6	0.288	0.859
Anion Gap	mEq/L	21.5	20.75	22.25	22	19.25	24	0.830	0.988

Clinically Ill and Pathogen Positive vs. Clinically Ill and Pathogen Negative:

Clinically ill cats that tested positive for pathogens had a lower platelet count ($n = 9$, $p = 0.0157$) than ill cats that were pathogen negative (Table 12). As each cat included had a platelet count below reference range, this difference is not likely to be a result of pathogen or clinical sign status. Plasma protein levels were above normal reference ranges for both groups, and slightly differed between them ($n = 9$, $p = 0.171$). Ill cats that were pathogen positive also had slightly lower lymphocyte percentage ($n = 9$, $p = 0.553$) than those who were ill and pathogen negative (Table 12). However, lymphocyte percentages were slightly below reference ranges for both groups. Median lymphocyte percentage differences were not statistically significant either before or after correction for multiple comparisons. No other CBC parameters differed significantly between compared groups.

Ill pathogen positive cats had lower BUN ($n = 11$, $p = 0.0853$) and ALT ($n = 11$, $p = 0.0576$) compared to ill pathogen negative cats (Table 13). Ill pathogen positive cats also had a higher total protein value ($n = 11$, $p = 0.0713$) than ill pathogen negative cats (Table 13). This value was above the provided reference range for ill pathogen positive cats, and within reference range for ill pathogen negative cats. Serum chloride concentrations were also slightly below reference range for ill pathogen positive cats and within reference range for ill pathogen negative cats. Due to a small sample size, no statistically significant differences between groups remained after correction for multiple comparisons.

Table 12. Comparison of CBC parameters between clinically ill cats who were pathogen positive and clinically ill cats who were pathogen negative. "↑" = above upper limit of reference range, "↓" = below lower limit of reference range. Bolded parameters were predicted to change with FURTD status.

		Ill Pathogen Positive Subjects (n=4)			Ill Pathogen Negative Subjects (n=5)				
Parameter	Unit of Measurement	Median	Q1	Q3	Median	Q1	Q3	p-value	Corrected p-value
WBC, total	**cells/µL**	**10240**	**9890**	**12210**	**10920**	**7880**	**12730**	**1**	**1**
RBC	**cells x 10_6/µL**	**8.495**	**7.71**	**9.05**	**9.060**	**9.01**	**9.4**	**0.413**	**0.961**
Hemoglobin	g/dL	12.65	11.8	12.875	13.6	13.3	13.6	0.176	0.961
Hematocrit	%	39.45	36.825	41.325	44.5	43.4	45	0.111	0.961
PCV	%	36.5	34.75	38	41	40	41	0.317	0.961
MCV	fl	47.2	46.525	47.55	47.9	47.1	49.4	0.556	0.961
MCH	pg	14.4	13.95	14.975	15.2	14.7	15.3	0.711	0.961
MCHC	g/dL	31.1	30.425	31.65	30.6	30.2	30.6	0.459	0.961
Red Cell Distribution Width	%	15.7	15.375	16.1	15.9	15.2	16.4	1	1
% Reticulocytes	%	0.45	0.375	0.55	0.5	0.5	0.6	0.814	1
# Reticulocytes	cells/µL	44250	36075	49525	43300	40100	44200	1	1
MPV	fl	20.2	17.5	23.4	19.4	17.3	20.7	0.806	1
Platelet Count	**x 1000 /uL**	**36 (↓)**	**31.25 (↓)**	**43.25 (↓)**	**229 (↓)**	**89 (↓)**	**238 (↓)**	**0.0157**	**0.393**
Plasma Protein	**g/dL**	**9.1 (↑)**	**8.9 (↑)**	**9.325 (↑)**	**8 (↑)**	**8 (↑)**	**8.1 (↑)**	**0.171**	**0.961**
% Neutrophils	**%**	**73**	**70.5**	**77.5 (↑)**	**71**	**67**	**81 (↑)**	**0.711**	**0.961**
% Lymphocytes	**%**	**16 (↓)**	**12 (↓)**	**19.75 (↓)**	**19 (↓)**	**19 (↓)**	**20**	**0.533**	**0.961**
% Monocytes	**%**	**3**	**2**	**5 (↑)**	**3**	**1**	**3**	**0.537**	**0.961**
% Eosinophils	%	4.5	3.5	5	3	3	4	0.620	0.961
% Basophils	%	0	0	0.75	0	0	0	1	1
% Bands	%	0	0	0	0	0	0	N/A	N/A
% Metamyelocytes	%	0	0	0	0	0	0	N/A	N/A
% Myelocytes	%	0	0	0	0	0	0	N/A	N/A
% Other	%	0	0	0	0	0	0	N/A	N/A
Nucleated RBC	#/100 WBC	0	0	0	0	0	0	N/A	N/A
# Neutrophils	**cells/µL**	**8122.5**	**7132.75**	**9850.25**	**5595**	**5166**	**8845**	**0.413**	**0.961**

# Lymphocytes	**cells/µL**	**1573.5**	**1174.5 (↓)**	**2375.75**	**2075**	**1542**	**2816**	**0.413**	**0.961**
# Monocytes	**cells/µL**	**373**	**312.75**	**491.75**	**282**	**236**	**382**	**0.556**	**0.961**
# Eosinophils	cells/µL	442	341.25	591	382	308	552	0.730	0.961
# Basophils	cells/µL	105	52.5	202 (↑)	0	0	0	0.306	0.961
# Bands	cells/µL	0	0	0	0	0	0	N/A	N/A
# Metamyelocytes	cells/µL	0	0	0	0	0	0	N/A	N/A
# Myelocytes	cells/µL	0	0	0	0	0	0	N/A	N/A
# Other	cells/µL	0	0	0	0	0	0	N/A	N/A
Neutrophil-Lymphocyte Ratio	**N/A**	**4.6996**	**3.53**	**6.714**	**3.737**	**3.35**	**4.263**	**0.730**	**0.961**

Table 13. Comparison of serum chemistry parameters between clinically ill cats who were pathogen positive and clinically ill cats who were pathogen negative. "↑" = above upper limit of reference range, "↓" = below lower limit of reference range. Bolded parameters were predicted to change with FURTD status.

		Ill Pathogen Positive Subjects (n=4)			Ill Pathogen Negative Subjects (n=7)				
Parameter	Unit of Measurement	Median	Q1	Q3	Median	Q1	Q3	p-value	Corrected p-value
BUN	mg/dL	21.5	19.75	23.25	24	23.5	25.5	0.0853	0.512
Creatinine	mg/dL	1.25	1.15	1.325	1.1	1	1.8	1	1
Glucose	mg/dL	93	85	98.5	72	69.5	85.5	0.315	0.945
Cholesterol	mg/dL	158.5	122.25	230.25 (↑)	172	159.5	204.5 (↑)	0.927	1
Total Protein	**g/dL**	**8.4 (↑)**	**7.775**	**9.025 (↑)**	**7.2**	**7.05**	**7.95**	**0.0713**	**0.512**
Albumin	g/dL	3.15	2.825	3.3	3.3	3	3.3	0.617	1
Bilirubin, Total	mg/dL	0.1	0.1	0.15	0.2	0.1	0.3	0.409	1
CK	U/L	236.5	176.5	344 (↑)	237	175.5	302.5	0.788	1
Alkaline Phosphatase	U/L	18	17.5	19.5	19	17	21	0.848	1
GGT	U/L	0 (↓)	0 (↓)	0 (↓)	0 (↓)	0 (↓)	0 (↓)	N/A	N/A
ALT (SGPT)	U/L	34.5	31.5	37.75	47	42	47.5	0.0576	0.5118
Sodium	mEq/L	151.5	150.75	152	150	149	151	0.287	0.945
Potassium	mEq/L	5.1	4.85	5.875 (↑)	4.9	4.8	5.15	0.632	1
Chloride	mEq/L	115 (↓)	113.75 (↓)	116.75 (↓)	117	115 (↓)	118.5	0.569	1
Calcium	mg/dL	9.45	8.15	9.6	9.1	8.95	9.65	1	1
Phosphorus	mg/dL	4.85	4.65	5.075	5	4.8	5.35	0.702	1
tCO2	mEq/L	19.8	18.7	21.1	18.5	18.05	19.15	0.315	0.945
Anion Gap	mEq/L	21.5	20.75	22.25	20	18.5	22	0.445	1

Urine and Fecal Testing—

Urinalyses were performed on 2 of 11 ill subjects and 2 of 7 healthy subjects; 4 cats in total. One cat had dilute urine (USG 1.016) and a creatinine level of 2.7 mg/dL, which is indicative of renal disease (International Renal Interest Society (IRIS) stage 2). However, as renal disease does not typically result in a systemic inflammatory response, this cat was not excluded from the clinicopathologic analysis. All other urinalysis parameters for the four tested cats were within normal limits. All cats tested negative for FeLV, FIV and Heartworm. Fecal examinations were performed for 9 of the 18 cats; all were negative for any ova or parasites. Fecal samples were not available for the remaining nine cats.

Discussion:

The primary goal of this pilot study was to compare clinicopathologic parameters between multiple groups of cats: cats with clinical signs of FURTD vs. cats without signs of FURTD, cats that tested positive for at least one of five common FURTD pathogens vs. those that did not, and ill cats that tested positive for pathogens vs. ill cats that tested negative for pathogens. We expected to see differences in parameters between groups, particularly in those involved in inflammatory responses, as cats with FURTD are often in a state of chronic inflammation. However, this was not necessarily the case in most comparisons. Clinical histories and physical exam findings were also analyzed during the course of the research.

Several cats in this study had discrepancies between owner- and veterinarian- reported signs, particularly concerning the presence of oral disease. Five of the eleven clinically ill cats had FURTD signs related to the oral cavity (gingivitis, stomatitis and/or faucitis), but only two owners reported oral inflammation. This is expected, as owners are not generally attuned to changes in feline oral health due to difficulty in visualization. More obvious ocular and nasal signs of FURTD tended to be detected by both owners and veterinarians, but not in all cases. Subjects nine and ten were both deemed non-clinical by their owner, but signs were apparent upon physical examination. Subject nine presented with ocular (discharge), oral (gingivitis). Stertor was also present, but was related to stenotic nares and therefore not a sign of FURTD. Subject ten presented with ocular (discharge and crusts) and oral (gingivitis and stomatitis) disease. Both subjects (9 and 10) tested positive for multiple FURTD pathogens (Table 6). These cats were also from the same multi-cat household, which can often make detection of disease more difficult for owners. These cats also had higher potential exposure to FURTD pathogens than other participating cats, as their owner works in a shelter environment and fosters kittens regularly. So, although these cats tested positive for respiratory pathogens, they did not necessarily contract these pathogens from one another. These discrepancies in detection of clinical signs provide insight into the importance of routine, thorough physical examination, as subtle signs of disease may be easily missed by owners.

The results of the qPCR respiratory pathogen testing were not consistent with expectations. While we expected to see agreement between the clinical presentation of respiratory signs and qPCR results, this was not the case for almost half of the participating subjects. The majority of cats that displayed clinical signs associated with FURTD tested negative for respiratory pathogens. Thus, cats that display FURTD signs do not necessarily have common respiratory pathogens. However, the validity of negative results via qPCR may be called into question, especially when considering possible sampling error due to intermittent mucosal shedding of tested respiratory pathogens (e.g. FCV, FHV-1). Additionally, the qPCR test results demonstrated that cats in the same household do not always share the same respiratory pathogens. This raises interesting questions regarding the transmission process of these pathogens, as well as what role the immune response or even the composition of the nasal microbiome plays in the prevention of infection. Despite these discrepancies, respiratory pathogen testing is still a valuable diagnostic tool; pathogen identification is an important step in developing a treatment protocol. However, while not currently commercially available or financially feasible for most owners, description of the upper respiratory microbiome structure and function (e.g. 16S rRNA gene sequencing and metatranscriptomic analysis) may be a more accurate approach to aid in the diagnosis of FURTD in comparison to the description of the species composition alone.

In general, both presence of clinical FURTD signs and FURTD pathogens seemed to be weakly associated with changes in inflammatory cell counts. For instance, cats with clinical signs and/or pathogens tended to have higher neutrophil counts and percentages in comparison to their clinically healthy and pathogen negative counterparts. This matches the initial hypothesis of the study, and aligns with expected physiological responses to chronic inflammatory conditions. As neutrophils are generally deployed to combat pathogens, we would expect to see increases in cats that are battling infection and exhibiting signs of inflammation. Interestingly, lymphocyte counts and percentages seemed to slightly decrease with presence of clinical signs or pathogen presence. In fact, the median lymphocyte percentages for both clinically ill cats and pathogen positive cats fell just below reference ranges provided by the OVDL. Lymphopenia has historically been associated with cases of viral infection, although usually FIV is the cause [21]. Theoretically, replication of upper respiratory viruses could result in host cell death via lysis, resulting in low lymphocyte populations. However, lymphopenia has not yet been linked to upper respiratory viruses in feline veterinary literature.

Neutrophil-lymphocyte ratios also differed between groups in two of the three comparisons performed (ill vs. healthy cats and pathogen positive vs. pathogen negative). To date, NLRs have been studied heavily in human medicine as a significant predictor of various inflammatory conditions (e.g. metabolic syndrome, thyroid diseases), as well as a prognostic factor in cases of sepsis and several types of cancer (e.g. breast and ovarian cancers) [22,23]. In feline medicine, NLR changes have been primarily used to asses inflammation associated with local recurrence of injection-site sarcomas and to predict recurrence risks. During the aforementioned study, elevated NLRs were associated with more advanced states of disease and systemic inflammation [20]. Because NLRs have only recently become the subject of veterinary research, reference intervals for cats have not yet been clearly established. However, trends in this study supported correlations between elevated NLRs and inflammatory conditions. For instance, cats with clinical signs of FURTD had a median NLR that was 1.886 units higher than cats without clinical signs. Similarly, cats that tested positive for FURTD pathogens had a median NLR that was 1.607 units above that of pathogen negative cats. Although a small sample size limited the statistical significance of this find, the trend is quite interesting and deserving of further exploration in the context of a larger study.

Total protein values were elevated for 5 of the 18 cats participating in this study. Of those five, four presented with FURD signs and one was considered clinically healthy. This was reflected in the differences in median total protein values between ill and healthy cats. Because total protein levels increase with both chronic inflammation and dehydration, the cause of the elevation in ill cats is difficult to assess. However, it's important to note that inflammatory conditions may result in dehydration, as the body produces excess mucous during these states.

Although correlations between clinicopathological inflammatory markers and FURTD disease state were weak within this study, there is a clear possibility of more evident associations within the general domestic feline population. Because this research was performed using a study population recruited for a pilot project examining respiratory microbiomes, inclusion criteria were relatively strict and resulted in a small sample size. Thus, one of the notable barriers faced during this project was achieving statistical significance for our findings. Further research to evaluate trends between clinicopathological inflammatory markers and FURTD disease state is indicated, and would benefit from a larger, more diverse study population.

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