Eguchi-Coe Y, Shiau D, Ma A et al. Integrative treatment combining *Yin Qiao San* with doxycycline for feline upper respiratory tract infection in shelter cats: A randomized, blinded, controlled clinical trial. Am J Trad Chin Vet Med 2024; 19(2):35-44. https://doi.org/10.59565/001c.121504

Clinical Studies

Integrative Treatment Combining *Yin Qiao San* with Doxycycline for Feline Upper Respiratory Tract Infection in Shelter Cats: A Randomized, Blinded, Controlled Clinical Trial

Yuko Eguchi-Coe*, Deng-Shan Shiau, Aituan Ma, Min Su Kim

ABSTRACT

The efficacy of integrating the Chinese herbal medicine, *Yin Qiao San* (YQS), with doxycycline compared to doxycycline alone for treating feline upper respiratory infection (URI) was investigated. Thirty-five cats exhibiting clinical signs of URI were randomly assigned to the Control or Test groups. All cats received 5 mg/kg dose of doxycycline. The Test Group additionally received YQS (dosed 50-100 mg/kg). All treatments were given twice daily for 10 days. Based on a 10-parameter URI clinical sign scoring system, improvement during the treatment period was analyzed. A subject was considered recovered from URI when scoring 0s on all signs for three consecutive days. Those who did not recover within 10 days continued having clinical sign scores assessed until recovery. In the Test Group, the mean recovery time was 9.3±3.6 days, which was significantly shorter (p=0.008) than the Control Group (14.0±6.0 days). The proportion of subjects recovered within 2 weeks was 94% (16/17) in the Test Group, which was significantly greater (p=0.002) than that in the Control Group (8/18=44%). Polymerase chain reaction (PCR) testing was performed to determine pathogens present on Day 1. The two most common pathogens identified were feline herpesvirus-1 and *Mycoplasma felis*. No cat tested positive for influenza A or influenza H7N2. Co-infection with more than 1 pathogen was present in 22% of controls and 41% of the Test Group. These findings suggest that integrating YQS with doxycycline resulted in quicker recovery than doxycycline alone when treating cats suffering from feline infectious respiratory disease complex.

Keywords: bordetella, chlamydia, doxycycline, feline calicivirus, feline herpesvirus-1, feline upper respiratory infection, herbal medicine, mycoplasma, PCR, *Yin Qiao San*

ABBREVIATIONS: FHV-1: feline herpesvirus-1; HHS: Heartland Humane Society; lb: pound(s); PCR: polymerase chain reaction; TCM: traditional Chinese medicine; URI: upper respiratory infection; YQS: *Yin Qiao San*

Upper respiratory infection (URI) often spreads readily between cats, especially in multi-cat environments, and can lead to significant morbidity for affected cats.[1] Several pathogens are believed to be responsible for most cases of URI in animal shelters. These include feline herpesvirus-1 (FHV-1), feline calicivirus, *Mycoplasma felis*, *Bordetella bronchiseptica*, *Chlamydophila felis*, and although not as common, influenza viruses.[1] Although there are available vaccinations for feline URI, respiratory tract disease continues to plague feline patients, especially those living in a multi-cat environment.[2]

Feline URI is rarely fatal in older populations; however, it is an important cause of morbidity and mortality in kittens.[3] In general, kittens are most susceptible to the infectious organisms that make up this complex disease, due to their lack of protective immunity from maternal antibodies which have decreased to insufficient levels or from vaccination failure. Unvaccinated adult cats are also at high risk for infection, and even vaccinated cats are at risk due to currently available vaccines providing only partial protection, although disease severity is reduced. Added to this, the establishment of subclinical/persistent

Author Professional Degrees and Certifications: DVM, MS, MS-TCVM, CVA, CVAT, CCRT, Elite FFCP, CVMMP, CTPEP, CVCH, CVFT, CVTP, CVBMA, CTCVMP, CVPP, CPEV (Eguchi-Coe); PhD (Shiau); DVM, PhD (Ma); DVM, PhD (Kim); **From:** West Hill Animal Hospital, Corvallis, Oregon, USA (Eguchi-Coe); Chi University, Reddick, Florida, USA (Eguchi-Coe, Shiau); Hebei Agricultural University, Baoding, Hebei Province, China (Ma); College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea (Kim); ***Address correspondence to** Dr. Eguchi-Coe (yukocoe@gmail.com).

infection in cats provides a constant source of pathogens back into the multi-cat environment. Poor husbandry which includes substandard housing conditions, lack of population management strategies, and sanitation breaks; all increase the infectious dose of pathogens for URI.[4]

Clinical signs of feline URI can vary considerably and are characterized by, but not limited to lethargy, inappetence, sneezing, conjunctival hyperemia, oral ulceration, hypersalivation, serous/mucopurulent nasal and ocular discharge. In severe cases there is progression to invasion of the lower respiratory tract, bronchopneumonia and death from respiratory distress.[3,5] Feline URI clinical signs can be acute (up to 10 days) or chronic (> 10 days).[3] The most important aspect of treatment for cats with URI is supportive care, which includes nutritional and nursing support. The cats are often anorexic because of their inability to smell due to nasal congestion, oral ulcer pain, and systemic illness. In severe cases, an esophagostomy tube may be placed to facilitate maintenance of necessary caloric intake. Parenteral crystalloid fluid administration may be required to maintain hydration and to reduce fever.[6]

Antibiotic therapy is beneficial to address primary disease (i.e. chlamydia, bordetella, mycoplasma) and/or secondary bacterial infections. Doxycycline (10mg/kg/day) has been recommended by the antimicrobial guidelines working group of the International Society for Companion Animal Infectious Diseases (ISCAID)[a] as the first choice of antibiotics for treating URI in cats.[5] It is effective against *Chlamydophila felis*, *Bordetella bronchiseptica*, and *Mycoplasma felis*, with good penetration of a patient's airway.[6] If successful treatment is not accomplished with the first tier antibiotic, current standard URI treatment recommends switching antibiotics. Also viral therapy should be considered. It has been reported that famciclovir reduces conjunctival FHV-1 shedding, which suggests that antiviral therapy could potentially play a role in interrupting the infectious cycle within cats with URI signs.[7] The generally inadequate response to conventional treatment of feline URI complex, particularly in multi-cat environments, accents the need to find more effective treatment strategies for these feline patients.

In traditional Chinese medicine (TCM), *Yin Qiao San* (YQS) is a classical herbal medicine formula used to treat respiratory disease. It was first introduced in *Systematic Differentiation of Warm Pathogen Diseases* by Wu Ju-Tong in 1798.[8] The herbal medicine formula was named after the two King herbs, *Jin Yin Hua* (Lonicera) and *Lian Qiao* (Forsythia) (Table 1).[9,10] From a TCVM perspective, upper respiratory infection is associated with Wind-Heat, Wind-Cold and Damp-Heat. Wind and Cold invade the body through the nose causing serous discharge. If *Wei Qi* is unable to repel the surface invasion, these pathogens invade deeper and enter the Lung and transform into Wind-Heat. Clinical signs then change from mild serous discharge to thick yellow exudate accompanied by fever. The pulse is usually strong/rapid, and the tongue is red-dry, reflecting the Excess Heat.[9]

Yin Qiao San is often used by integrative veterinary medicine clinicians to treat the TCVM Pattern of Wind-Heat invasion, which is an important and commonly diagnosed pattern in early-stage feline upper respiratory infections.[9-12] The primary actions of this herbal medicine formula are to expel exogenous pathogens, clear Heat, and eliminate toxins. In traditional Chinese medicine (TCM), *Yin Qiao* Powder is usually the first prescription prescribed to humans for upper respiratory disease due to its excellent reputation as an exterior-resolving prescription.[8] *Yin Qiao San* has been shown to have good effects on the mouse model of upper respiratory mucosal immunity dysfunction induced by cold stimulation with bacteria and viruses. In addition to its antibacterial and antiviral effects, it is considered to contribute to the improvement of upper respiratory mucosal immune system function.[8]

The objective of this study was to determine whether integrating the Chinese herbal medicine formula, YQS, with doxycycline could reduce the recovery time of URI cats when compared to treatment with doxycycline alone. Through a randomized blinded controlled trial, the study aimed to test the hypothesis that subjects treated with both YQS and doxycycline would have shorter recovery time than those only treated with doxycycline. Secondary metrics gathered and analyzed, included the presence and prevalence of pathogens in study animals at the start of the study.

Table 1: Ingredients of the Chinese herbal medicine *Yin Qiao San* and their actions[10]

***Pin Yin* Name**	**English Name**	**Actions**
Jin Yin Hua	Lonicera	Clears Heat Toxin, clears the Exterior
Lian Qiao	Forsythia	Clears Heat and Toxins, subdues swelling
Ban Lan Gen	Isatis	Clears Heat Toxins
Lu Gen	Phragmites	Clears Heat and promotes Body Fluids
Bo He	Mentha	Disperses Wind-Heat
Niu Bang Zi	Arctium	Disperses Wind-Heat in the Lung Channel, relieves sore throat
Jie Geng	Platycodon	Opens the Lung *Qi*, relieves cough, dissolves Phlegm
Jing Jie Sui	Schizonepeta	Opens the surface to relieve Exterior pathogen
Dan Zhu Ye	Lophatherum	Clears Heat, relieves restlessness, promotes diuresis
Sheng Gan Cao	Glycyrrhiza	Harmonizes, treats sore throat, generates fluids

MATERIALS AND METHODS

Animals

Subjects were recruited from Heartland Humane Society (HHS), located in Corvallis, Oregon. During the spring and summer months, the shelter receives upward of 50-70 cats (including kittens), with most admitted cats showing clinical signs of URI. These cats provided the subject population that was considered for enrollment in the study. All of HHS's cats with URI signs were immediately placed into an isolation room when clinical signs were noted. Subjects meeting the following criteria were enrolled in the study: (1) cats of any age and either sex; (2) cats showing one or more clinical signs of URI (e.g. sneezing, conjunctival hyperemia, serous/mucopurulent nasal and ocular discharge, hypersalivation/oral ulceration); and (3) newly diagnosed URI without previous treatment history. Exclusion criteria included: (1) cats weighing less than 0.9 kg (2 lb), as weighing and preparing such a small amount of herbal medicine may be inaccurate; and (2) feral cats that could not be handled.

Treatment Protocol

Subjects that qualified for enrollment were randomly assigned into two treatment groups: Control or Test. Subjects in the Control Group were treated with doxycycline only, and those in the Test Group received doxycycline and YQS. Group randomization was conducted using a commercial computer software[b] program which provided a random number generator for group assignment.

Subjects in both groups received a liquid compounded solution (50 mg/mL) of doxycycline[c] (oral dose, 5 mg/kg, twice daily). The Test Group cats also received a capsule of YQS[d] containing a weighed amount of the test herbal formula powder (oral dose, 50-100 mg/kg, twice daily). This was followed by a bolus of water. All of the assigned treatments were given by veterinary assistants twice daily for 10 days.

During the trial, any patient with fever over 102 °F also received 10 mL/lb subcutaneous (SQ) fluid. All medications were set up by the veterinarian on staff in blinded medication envelopes (i.e. non-transparent paper envelope). Hence, the assistants medicating the cats were blinded to the group assignments to prevent potential procedural bias. The Test Group received doxycycline and an appropriate YQS dose capsule, and the Control Group received doxycycline and an empty capsule. Medications were given to the patients before feeding and cleaning of their environment, followed by the URI assessments. The cats were treated for all 10 days of the study, even if they had left isolation.

After the enrollment and before treatment started, each subject had nares and pharynx samples taken for polymerase chain reaction (PCR) testing to determine the pathogens present at the start of treatment. Samples were sent to a commercial laboratory[e] for a feline respiratory pathogen diagnostic panel: *Chlamydophila felis*, *Bordetella bronchiseptica*, *Mycoplasma felis*, feline herpesvirus-1, feline calicivirus, feline H7N2 influenza virus, influenza A.

Outcome Data and Statistical Analysis

Subject signalment data including age, sex, breed, and body weight were collected at the time of enrollment for group comparability analysis. Before treatment started, all subjects had nares and pharynx samples taken for PCR testing to determine the pathogens present on Day 1. A subject's URI clinical signs were assessed using the scoring system that had been previously set up at this local shelter. The 10-parameter scoring system, used to calculate the overall sum of URI clinical signs presented by a sick animal, was a modification of a published URI grading system for shelter cats (Table 2).[13] The assessments were repeated daily for at least 10 days after the treatments started. Based on the 10-parameter URI score, a subject was considered recovered when scoring 0s on all clinical signs for three consecutive days and after being authorized by an attending veterinarian (blinded to the study) to leave isolation. Cats that left isolation before 10 days continued receiving treatment and receiving URI clinical sign scores through the 10th day. Those who did not recover after 10 days continued having the URI clinical sign scores assessed until recovery.

There were two sets of statistical hypotheses tested: 1) comparison of the 2-week recovery rate between groups; 2) comparison of mean improvement of the overall clinical sign score at the end of the 10-day treatment period between groups. For the first hypothesis, the study tested the null hypothesis (H_0) that the 2-week recovery rate among the Test Group population was the same as that among the Control Group population versus the alternative hypothesis (H_A) that the Test Group had a greater recovery rate. One-sided Fisher's Exact test was applied to test the hypotheses and the H_0 was rejected if the *p*-value was less than 0.05. The second hypothesis tested that the H_0 at the end of the 10-day treatment period had a mean improvement (or reduction) of the overall clinical sign score among the Test Group population that was the same as that among the Control Group population, versus the H_A that the Test Group has greater mean improvement. Without assuming normality, the non-parametric one-sided Wilcoxon Rank Sum test was applied. The H_0 was rejected if the resulting *p*-value was less than 0.05. In addition, the within-group improvement in each treatment group was evaluated using simple regression analysis to estimate the rate of improvement (per day) over the 10-day treatment period.

The study planned to enroll a total of 46 URI cats, 23 in each group, which would provide approximately 88% power for Fisher's Exact test (assuming 90% and 50% within-2-week recovery rate for the Test and Control groups, respectively) and 94% power for the Wilcoxon test (assuming an effect size=group difference/standard deviation=1.0) to reject the null hypotheses with a 0.05 significance level. A commercial software system for statistical computing and graphics was used for all data graphic presentations and statistical analyses[f].

Table 2: Scoring system used to grade the severity of upper respiratory infection clinical signs in study cats

Location	Clinical Signs		Points Allotted
Eyes	No ocular involvement		0
	Conjunctivitis	Serous conjunctivitis	1
		Mucopurulent (green/yellow) or bloody discharge and moderate to marked conjunctivitis	2
	Corneal ulcers/Keratitis		3
Nose	No nasal discharge, sneezing (ocular signs only)		0
	Sneezing	Serous (clear) nasal discharge and sneezing	1
		Mucopurulent (green/yellow, bloody) nasal discharge and sneezing	2
		Large amount thick green discharge and sneezing	3
Mouth	No oral involvement		0
	Salivation		1
	Ulcers	Moderate to marked inflammation	1
		One oral ulcer <3mm in diameter	2
		Multiple ulcers or one ≥3mm in diameter	3
		Bleeding ulcer	4
Hydration	Adequately hydrated		0
	Dehydration	Slight dehydration (mildly tacky gums, slow return of tented skin)	1
		Severe dehydration (dry gums, sunken eyes, slow return of tented skin to normal state)	2
Lungs	Congestion	None	0
		Possible congestion/Coughing	1
		Audible chest sounds (crackles on auscultation)	2
		Labored or open mouth breathing (dyspnea)	3
Attitude	BAR	Bright, alert, responsive	0
	QAR	Quiet, alert, responsive	1
	L	Lethargic	2
	U	Markedly unresponsive	3
Appetite	Eating	Normally	0
		Eating less than expected (Fecal output <1x per day)	1
		Not eating	2
Drinking	Drinking	Normally	0
		Less than expected (Urine output <2x per day)	1
		Not drinking	2
Weight	Stable weight		0
	Gaining weight		0
	Weight loss		1
Fever (°F) or Subnormal Body Temp	100 – 102		0
	102 – 103.5 or subnormal temperature 99 – 100		1
	>103.5 or subnormal temperature <99		2

RESULTS

The study was able to enroll a total of 35 cats that met the inclusion/exclusion criteria during the study timeline. A total of 18 cats were randomly assigned to the Control Group and 17 to the Test Group. All subjects completed the study with no adverse effects documented for either study group. The smaller than planned sample size provided a 78% power for the recovery rate comparison (Fisher's Exact test) and an 88% power for the URI score improvement comparison under the same statistical considerations.

Subject signalment data including age, body weight and sex proportions were analyzed and compared between groups to confirm group comparability for the study. There were 9 cats in the Test Group older than 1 year of age with 1 animal over 10 years old (mean±SD = 3.12±2.9). In the Control Group there were 11 patients over 1 year of age, also with 1 cat older than 10 years old (mean±SD = 3.1±3.5). The group difference in a subject's median age was not statistically significant (p = 0.725). There were 15 cats in the Test Group with a body weight over 2.25 kg (5 lb) and 2 cats below 2.25 kg (5 lb), (mean±SD = 9.1±3.6). The Control Group was similar with only 2 cats under 2.25 kg (5 lb) (mean±SD = 8.2±3.0). Similarly, the group difference in subject's median body weight was not statistically significant (p = 0.347). With regard to sex proportion, the Test Group had 6 intact males and 6 castrated males (12/17 = 70.6%). There were 4 spayed female cats and 1 intact female in the Test Group (5/17 = 29.4%). In the Control Group, there were 13 male cats (13/18 = 72.2%) made up of 7 intact males and 6 castrated males. Five female cats were in the Control Group (5/18 = 27.8%) made up of 3 intact females and 2 spayed females. Statistically, the sex distribution was not significantly different between the two treatment groups (p = 1.00).

Based on the 10-parameter URI clinical sign scoring system and the attending veterinarian's assessment, the Test Group had a mean±SD recovery time of 9.3±3.6 days, compared to 14.0±6.0 days in the Control Group (Figure 1). Statistically, the median recovery time in the Test Group was significantly shorter than that in the Control Group (p = 0.008). When considering 14 days as the maximal acceptable recovery time, there were 16 out of 17 (94.1%) cats in the Test Group recovered within the threshold, whereas only 44.4% (8/18) of the cats in the Control Group met the 14-day recovery threshold (Figure 2). Statistically, the 2-week recovery rate in the Test Group was significantly greater than that in the Control Group (p = 0.002).

Daily mean overall clinical scores based on the 10 parameters scoring system (i.e. sum of the 10 scores) within each treatment group were estimated (Figure 3 and Table 3). Before initiation of the assigned treatment (i.e. Day 1), the overall clinical sign scores were not significantly different between the two treatment groups (mean±SD: Control 4.2±1.7 vs. Test 3.8±1.3; p = 0.610). Both groups' mean clinical scores improved over time (all p < 0.001) with overall improvement for controls at 0.28 per day (p < 0.001) and 20% greater for test cats at 0.33 per day; group comparison (p = 0.145). Findings for the 10 individual clinical sign scores suggested that, in both treatment groups, clinical signs for eyes, nose, and lungs contributed the most to the overall score improvements (Day 10 compared to Day 1, Table 4).

The presence of infectious organisms, including *Chlamydophila felis*, *Bordetella bronchiseptica*, *Mycoplasma felis*, FHV-1, feline calicivirus, feline H7N2 influenza, and influenza A were tested before treatment (Table 5). The mean number of infectious organisms (per cat) for the Control Group was 1.0±1.0 (mean±SD) while the Test Group was slightly higher at 1.4±0.8 (p = 0.158). The most prevalent organism documented by PCR for the study was FHV-1 (19/35, 54% of all cats). This was followed by *Mycoplasma felis* (12/35, 34%), and then *Chlamydophila felis* (4/35, 11%), feline calicivirus (4/35, 11%), and *Bordetella bronchiseptica* (2/35, 6%).

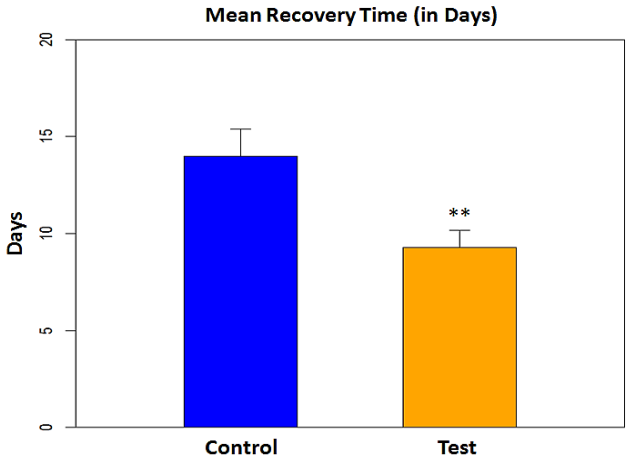

Figure 1: Mean recovery time (in days) within each treatment group. ** p < 0.01 smaller than the Control Group.

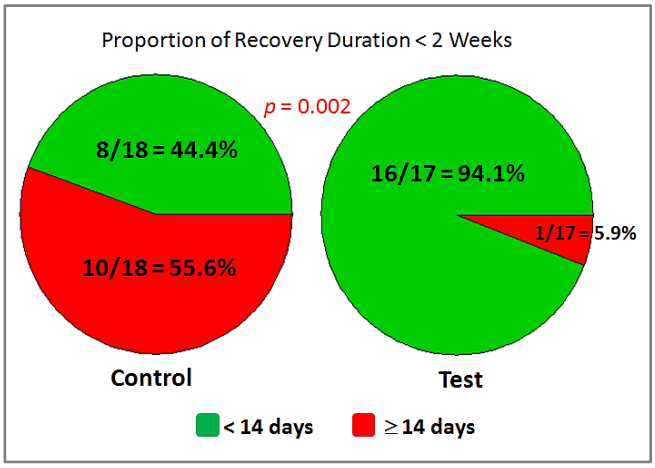

Figure 2: Proportion of recovery time within 2 weeks within each treatment group.

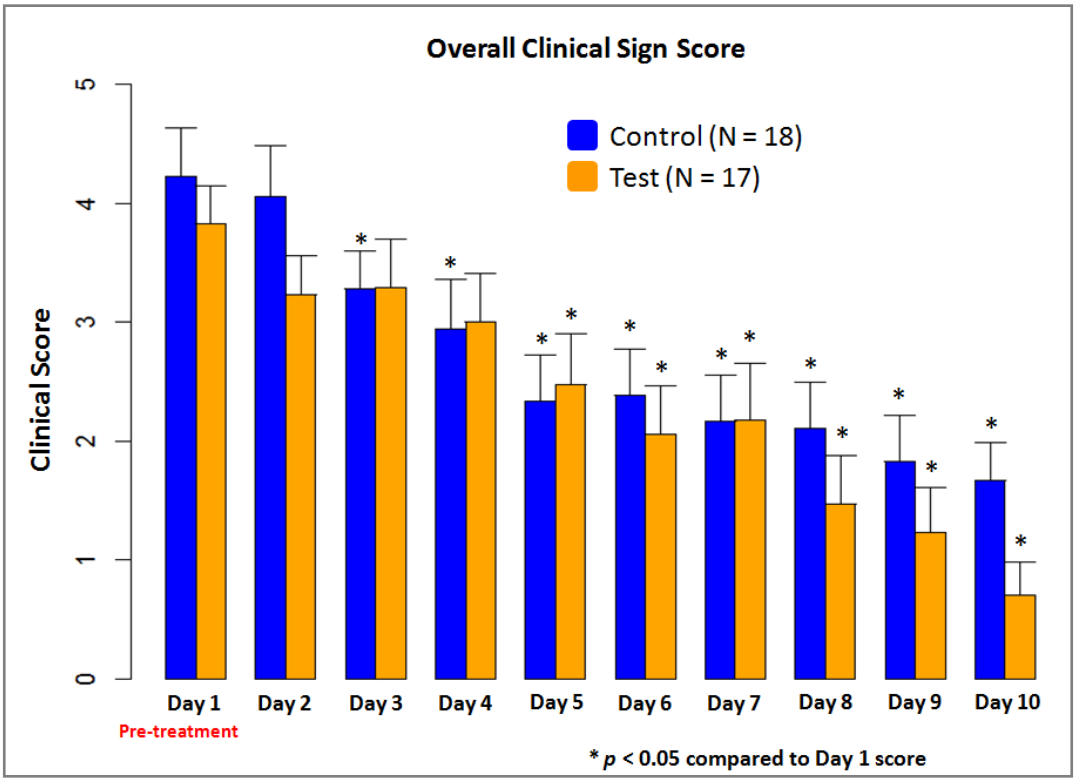

Figure 3: Mean overall clinical sign score improvement within each treatment group from Day 1 (pre-treatment baseline) to Day 10

Table 3: Summary statistics (mean±SD) for intragroup comparison of URI clinical sign score improvement in each treatment group from Day 1 to Day 10

	Control Group (improvement from Day 1)	Test Group (improvement from Day 1)	Control vs. Test on Improvement (*p*-value)
Day 1	4.22±1.73	3.82±1.33	NA
Day 2	4.06±1.80 (0.17±1.20) 3.9%	3.24±1.35 (0.59±1.18) 15.4%	0.246
Day 3	3.28±1.36 (0.94±1.59) 22.4%	3.29±1.65 (0.53±1.23) 13.8%	0.676
Day 4	2.94±1.76 (1.28±1.99) 30.2%	3.00±1.70 (0.82±1.67) 21.5%	0.665
Day 5	2.33±1.64 (1.89±2.19) 44.7%	2.47±1.77 (1.35±1.46) 35.4%	0.654
Day 6	2.39±1.61 (1.83±2.57) 43.4%	2.06±1.68 (1.76±1.64) 46.2%	0.772
Day 7	2.17±1.65 (2.06±2.62) 48.7%	2.18±1.98 (1.65±1.87) 43.1%	0.908
Day 8	2.11±1.64 (2.11±2.63) 50.0%	1.47±1.70 (2.35±1.90) 61.5%	0.248
Day 9	1.83±1.62 (2.39±2.52) 56.6%	1.24±1.56 (2.59±1.70) 67.7%	0.581
Day 10	1.67±1.37 (2.56±2.48) 60.5%	0.71±1.16 (3.12±1.32) 81.5%	0.291

Table 4: Treatment effect on individual clinical signs over the 10-day treatment period

Anatomy/Location	Control Group (n = 18)			Test Group (n = 17)		
	Improved	Worsened	No Change	Improved	Worsened	No Change
Eyes	9 (50%)	0 (0%)	9	12 (71%)	0 (0%)	5
Nose	12 (67%)	0 (0%)	6	14 (82%)	0 (0%)	3
Mouth	0 (0%)	0 (0%)	18	0 (0%)	0 (0%)	17
Hydration	5 (28%)	0 (0%)	13	0 (0%)	0 (0%)	17
Lungs	8 (44%)	2 (11%)	8	11 (65%)	0 (0%)	6
Attitude	5 (28%)	1 (6%)	12	4 (24%)	1 (6%)	12
Appetite	2 (11%)	0 (0%)	16	1 (6%)	0 (0%)	16
Drinking	1 (6%)	0 (0%)	17	0 (0%)	0 (0%)	17
Weight	0 (0%)	6 (33%)	12	1 (6%)	1 (6%)	15
Fever	5 (28%)	0 (0%)	13	2 (12%)	0 (0%)	15

No cats were positive for feline H7N2 influenza virus or influenza A virus. The percent presence of individual infectious organisms in each group was similar with the exception of *Mycoplasma felis*, where the Test Group had significantly more prevalence of the pathogen than the Control Group (p = 0.035), (Table 5). Four cats in the Control Group (4/18, 22.2%) were carrying co-infections of organisms, where the organisms were different among these cats, but 3 of them carried FHV-1 and *Chlamydophila felis*. Seven cats in the Test Group (7/17, 41.2%) had co-infections with varied organism combinations, but most of them (5/7) had FHV-1 and *Mycoplasma felis.* The Test Group had a non-statistically significant increase (almost 20%) of co-infected cats when compared to the controls (p = 0.289).

The recovery time and presence of FHV-1 or *Mycoplasma felis* (two most prevalent pathogens present), or co-infection of study cats was investigated. In the Control Group, the mean±SD recovery time among cats with FHV-1 infection was 13.8±5.5 days, while the Test Group cats infected by this organism had a mean of 11.3±2.5 days. For the *Mycoplasma felis* organism, the recovery time for the controls was 16.7±4.0 days, while the Test Group mycoplasma recovery was 9.0±4.2 days. Control Group cats that had co-infections had a mean recovery time of 15.0±6.1 days (longer than group mean recovery time of 14.0±6.0), while Test Group co-infected cats had a mean recovery of 11.7±2.1 days (longer than their group mean recovery of 9.3±3.6, Table 6).

Table 6: Length of recovery time for individual cats and infection with feline herpesvirus-1, or *Mycoplasma felis*, or co-infection with more than 1 infectious organism in the Control and Test groups

Infection (Positive Cats)	**Control Group** (Mean±SD)	**Test Group** (Mean±SD)
FHV-1	13.8±5.5	11.3±2.5
Mycoplasma	16.7±4.0	9.0±4.2
Co-infected	15.0±6.1	11.7±2.1

Table 5: Summary table of number of cats PCR positive for the presence of *Chlamydophila felis*, *Bordetella bronchiseptica*, *Mycoplasma felis*, feline herpesvirus-1, feline calicivirus, feline H7N2 influenza, and influenza A; also number of cats with co-infection of these organisms. Between-group comparisons are shown for each of the organisms and co-infection.

	Co-infection	**Feline Herpesvirus-1**	***Mycoplasma felis***	***Chlamydia felis***	**Feline Calicivirus**	***Bordetella bronchiseptica***	**Feline H7N2 Influenza virus**	**Influenza A virus**
Control (n = 18)	4 (22%)^	10 (56%)	3 (17%)	3 (17%)	1 (6%)	1 (6%)	0	0
Test (n = 17)	7 (41%)	9 (53%)	9 (53%)	1 (6%)	3 (18%)	1 (6%)	0	0
***p*-value**	0.289	1.000	0.035*	0.603	0.338	1.000	1.000	1.000

^ Number in parentheses = percent (rounded) of cats in group infected with organism; * = significant difference (p<0.05)

DISCUSSION

Feline infectious bacterial and viral respiratory disease complex causes widespread morbidity in shelter cats at tremendous cost to these institutions and decreases success for animal adoption. The objective of this study was to determine whether integrating a Chinese herbal medicine, *Yin Qiao San*, with an antibiotic could reduce recovery time for shelter cats affected with infectious respiratory disease complex. This prospective, randomized blinded controlled study found that cats with naturally occurring URI, treated with *Yin Qiao San* and doxycycline, had significantly shorter recovery time (9.3±3.6 days) than those that received only doxycycline treatment (14.0±6.0 days, $p = 0.008$). The mean difference in recovery was almost 5 days. In addition, 94% of the cats receiving the integrative treatment recovered within 2 weeks, while less than half (44%) in the Control Group achieved such a recovery time. Study findings supported the hypothesis that *Yin Qiao San* combined with doxycycline would result in quicker recovery for URI affected shelter cats, and with no report of adverse side effects associated with its administration to the 17 cats in the Test Group.

The improved URI recovery time in the present study, associated with the addition of YQS to doxycycline, is similar to Hirsch et al.'s study of shelter cats with naturally occurring URI in 2017.[9] A numerical clinical sign scoring system was devised with cats scored at study start and throughout the 10-day study. By Day 4, there was significant, within group, clinical score improvement ($p < 0.001$) in cats dosed with YQS and an antibiotic (doxycycline or Clavamox), compared to lack of significant improvement in antibiotic only cats. This statistical significance remained throughout the rest of the study. Additionally, the study showed that YQS was cost effective for use in shelters, and similar to the present study, also had no adverse side effects when administered to test animals.[9]

Both intragroup and intergroup comparison of the clinical signs score improvement, based on a 10-parameter scoring system similar to Hirsch et al, was evaluated in this study. At study initiation, the scores between groups were not significantly different. Over the study period, both groups had significant improvement with the Control Group having overall improvement of 0.28 per day ($p < 0.001$) and 20% greater for the test cats at 0.33 per day ($p < 0.001$). Although the test cats improved at a more rapid pace, it did not achieve statistical significance ($p = 0.145$). The lack of significance between groups for clinical sign improvement was unexpected as the recovery time difference between groups was significantly faster for the Test Group ($p = 0.008$). The authors suggest the statistical difference between recovery time and clinical sign score improvement is most likely related to the control animals' greater difficulty in maintaining 3 days of a "0" score to achieve "recovered" status. The control cats tended to have intermittent relapses where they would again demonstrate clinical signs for periods of time, in contrast to the test cats. This made it more difficult for controls to achieve recovered status, thus they had longer recovery times even though their clinical signs had statistically significant improvement.

A closer look at each of the 10 individual clinical sign scores suggested that, in both treatment groups, clinical signs for eyes, nose, and lungs contributed the most to the overall clinical sign score improvements (Day 10 compared to Day 1). Among the 17 subjects in the Test Group, 12 (71%) had an improvement for "eyes" clinical signs, 14 (82%) had an improvement for "nose" clinical signs, and 11 (65%) had an improvement for "lung" clinical signs. Similarly, in the Control Group, among the 18 subjects, 9 (50%) had improvement "eye" clinical signs, 12 (67%) had improvement "nose" clinical signs, and 8 (44%) had improvement "lung" clinical signs. It is worth noting that there were 6 subjects (33%) in the Control Group losing body weight, whereas only 1 (6%) Test Group subject had body weight loss. In addition, there were several observations from this study suggesting that feline URI patients treated with antibiotics and YQS appeared to feel better although they still experienced clinical signs of URI. Similar observations on patient's quality of life when treated with *Yin Qiao San* have been reported in human studies.[14]

In the present study, secondary metrics were gathered and analyzed on the presence and prevalence of pathogens at study start along with varying recovery times dependent on the pathogen. Study findings demonstrated that the most prevalent infectious organism in study cats was FHV-1 (19/35, 54%), followed by mycoplasma (12/35, 34%). Fewer cats had *Chlamydophila felis* (11%), feline calicivirus (11%), and *Bordetella bronchiseptica* (6%) and no cats were positive for feline H7N2 influenza or influenza A viruses. The percent presence of individual infectious organisms in each group was similar with the exception of *Mycoplasma felis*, where the Test Group had significantly more prevalence of the pathogen than the Control Group ($p = 0.035$). The mean number of infectious organisms (per cat) for the Control Group was 1.0±1.0 (mean±SD) while the Test Group was non-significantly higher at 1.4±0.8 ($p = 0.158$). Co-infections were documented in 22% and 41% of control and test cats, respectively; and mean recovery time was longer for these patients. The co-infected controls had a 15.0±6.1 days recovery time versus 14.0±6.0 day mean for the total Control Group, while co-infected test cats had an 11.7±2.1 day recovery versus 9.3±3.6 day mean for the total Test Group.

Case studies have shown that YQS has been used to successfully treat pneumonia in cattle, and upper respiratory infection in horses.[12,15] Reasons for its treatment success are being illuminated in mechanistic work. When investigating YQS's conventional actions (anti-inflammatory, anti-pyretic, antibacterial, anti-viral)

and mechanism of action, positive effects have been shown to be related to its ability to modify mucosal immune dysfunction by improving the upper respiratory tract mucosal immune system. Murine mechanistic studies support this and have documented YQS can increase macrophage phagocytic index and phagocytic coefficient, as well as act against the suppression of humoral immunity induced by cyclophosphamide.[8] In a mouse pneumonia model, a combined formula of *Xi Jiao Di Huang Tang* and YQS inhibited the production of inflammatory cytokines and free radicals in mice infected with influenza virus.[9]

Studies investigating the King herbs, *Jin Yin Hua* (Lonicera) and *Lian Qiao* (Forsythia) along with the Minister herbs *Jie Geng* (Platycodon), *Bo He* (Mentha), and *Niu Bang Zi* (Articum) demonstrate these same positive effects.[9,12] Both Lonicera and Forsythia have demonstrated marked anti-inflammatory effects in multiple studies investigating lipopolysaccharide induced lung inflammation.[9] Some of these effects include increased expression of IL-10 (important anti-inflammatory cytokine), while proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6, were decreased. Additionally, a major lignin (Forsynthin) found in Forsythia has been studied *in-vitro* and *in-vivo* in murine studies of bacterial pneumonia, sepsis and cytokine-driven inflammation. It was found that Forsynthin inhibits phosphodiesterase 4 (PDE4) in inflammatory and immune cells and has direct effects on the influenza A viruses (decreased viral titers, reduced influenza M1 protein limits viral spread).[9,16]

Lonicera, Forsythia and Platycodon were studied alone and combined in the treatment of experimental murine pulmonary inflammation. Study findings demonstrated that mice in the 3-herb combination group had the best results with statistically significant reductions of TGF-β, IL-1β and WBC counts. Further, TEF3-mRNA (trefoil factor 3 which protects respiratory mucosa) was significantly increased; leading the authors to conclude that Platycodon acts synergistically with the King herbs in YQS to reduce inflammatory cytokines and protect lung tissue.[9] Additional investigations have demonstrated activity against multiple viruses, as well as antibacterial, and anti-inflammatory activity for Mentha. Articum is similar with demonstration of multiple respiratory disease treatment benefits (e.g. anti-oxidant, antibacterial, anti-inflammatory, antifungal). Finally, *Fu Fang Yin Hua Jie Du* granules (i.e. optimized YQS formula) was used to treat influenza and upper respiratory tract infection associated with SARS in 2003. The modified YQS formula showed broad spectrum anti-influenza virus activity in vitro, and significant protective effect in mice against lethal influenza virus infection. Much of the beneficial effects were attributed to suppressing the expression of inflammatory cytokines.[14]

The delay of test cats demonstrating statistically significant clinical sign score improvement until Day 5 was unexpected. The Control Group had statistical significance by Day 3 and had better score improvement than the Test Group from Day 3 through Day 5. The authors suggest this delay may be related to the greater number of co-infected cats in the Test Group. It has been recognized in both human and veterinary medicine that viral infections predispose patients to bacterial infections and that co-infections have worse outcomes than either infection alone.[6,17] In humans, the lengthened disease recovery and poorer outcomes has been documented with multiple influenza pandemics as well as recently with SARS-CoV-2 (COVID-19) pandemic.[17] In veterinary medicine, particularly as associated with the feline respiratory disease complex, cats with co-infections demonstrate more severe disease with a more complicated clinical picture than single pathogen infection.[6] Commercial diagnostic labs performing feline respiratory diagnostic panels have reported a 45-48% co-infection rate.[6] This suggests that the percent of co-infected test cats (41%) for this study is consistent with the literature while the controls (22%) were less affected with this complication than would be expected. Simultaneous infection with multiple pathogens exacerbates the severity of illness, therefore, it is reasonable to conclude that increased co-infection of the test cats most likely slowed recovery for the first few days of the study.[6]

Limitations to this study included smaller than desired sample size for each treatment group and all animals sourced from only one shelter facility. The small sample size complicated by animal variability reduced statistical power, making statistically valid intergroup and intragroup comparisons difficult. Additionally, all URI subjects were enrolled from one shelter, which could affect the generality to the real-world feline URI population.

In summary, it was found naturally occurring URI in shelter cats treated with a combination of *Yin Qiao San* and doxycycline had a significantly shorter recovery time than cats given doxycycline only. Additionally, the proportion of subjects recovered within 2 weeks was 94% in the Test Group, which was significantly greater than that in the Control Group (44%). There were no adverse effects observed in test cats dosed with YQS and doxycycline. Studies with a larger sample size from multiple shelters as well as with a longer monitoring duration (> 10 days) of clinical scores are suggested to validate the findings of this study and further determine optimum treatment of upper respiratory disease complex in shelter cats.

ACKNOWLEDGMENTS

The authors would like to thank all the veterinary staff and volunteers at Heartland Humane Society for their teamwork and irreplaceable support with this research study. Sincere appreciation is also dedicated to the Chi University staff and master's thesis committee members for the invaluable opportunity and support to this project.

Declaration of Interest and Funding

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this paper and the authors did not receive any specific grant of funding from any organization in the public, commercial or non-profit sectors.

FOOTNOTES

[a] International Society for Companion Animal Infectious Diseases, www.iscaid.org
[b] GraphPad Software, Inc, www.graphpad.com
[c] NW Compounding Pharmacy, Tualatin, Oregon, USA
[d] Dr Xie's Jing Tang Herbal, Inc., Ocala, Florida, USA
[e] Idexx laboratory, One Idexx Drive, Westbrook, Maine USA
[f] R version 4.2.1. The R Foundation for Statistical Computing, Vienna Austria; http://www.R-project

REFERENCES

1. Bannasch M, Foley J. Epidemiologic evaluation of multiple respiratory pathogens in cats in animal shelters. J Feline Med Surg 2005; 7(2):109-119. https://doi.org/10.1016/j.jfms.2004.07.004
2. Dawson S, Willoughby K. Feline infectious upper respiratory tract disease - An update. In Practice 1999; 21(5):232-237. https://doi.org/10.1136/inpract.21.5.232
3. Sykes J. Pediatric feline upper respiratory disease. Vet Clin North Am Small Anim Pract 2014; 44(2):331-342. https://doi.org/10.1016/j.cvsm.2013.10.005
4. Dinnage J, Scarlett J, Richards J. Descriptive epidemiology of feline upper respiratory tract disease in an animal shelter. J Feline Med Surg 2009; 11(10):816-825. https://doi.org/10.1016/j.jfms.2009.03.001
5. Lappin M, Blondeau J, Boothe D et al. Antimicrobial use guidelines for treatment of respiratory tract disease in dogs and cats: antimicrobial guidelines working group of the international society for companion animal infectious diseases. J Vet Intern Med 2017; 31(2):279-294. https://doi.org/10.1111/jvim.14627
6. Cohn L. Feline respiratory disease complex. Vet Clin North Am Small Anim Pract 2011; 41(6):1273-1289. https://doi.org/10.1016/j.cvsm.2011.07.006
7. Reinhard C, McCobb E, Stefanovski D et al. A randomized, placebo-controlled clinical trial of famciclovir in shelter cats with naturally occurring upper respiratory tract disease. Animals 2020; 10(9):1448-1460. https://doi.org/10.3390/ani10091448
8. Liu L, Lei N, Lin Q et al. The effects and mechanism of *Yinqiao* powder on upper respiratory tract infection. Int J Biotechnol Wellness Ind 2015; 4(2):57-60. http://dx.doi.org/10.6000/1927-3037.2015.04.02.2
9. Hirsch D, Shiau D, Xie H. A randomized and controlled study of the efficacy of *Yin Qiao San* combined with antibiotics compared to antibiotics alone for the treatment of feline upper respiratory disease. Am J Trad Chin Vet Med 2017; 12(2):39-47. https://doi.org/10.59565/001c.120314
10. Ma A. Clinical Manual of Chinese Veterinary Herbal Medicine 4th Ed. Gainesville, FL: Ancient Art Plus 2016:220-221.
11. Dashtdar M, Dashtdar M, Dashtdar B et al. The concept of wind in traditional Chinese medicine. J Pharmacopuncture 2016; 19(4):293-302. https://doi.org/10.3831/KPI.2016.19.030
12. Song D, Schmalberg J, Xie H. Herbal formulas to release the Exterior. Xie's Chinese Veterinary Herbology. Xie H, Priest V (eds). Ames, IA: Wiley Blackwell 2010:352-353.
13. Lappin M, Sebring R, Porter M et al. Effects of a single dose of an intranasal feline herpesvirus 1, calicivirus, and panleukopenia vaccine on clinical signs and virus shedding after challenge with virulent feline herpesvirus 1. J Feline Med Surg 2006; 8(3):158-163. https://doi.org/10.1016/j.jfms.2005.12.001
14. Zhang Y, Wang R, Shi W et al. Antiviral effect of *fufang yinhua jiedu* (FFYH) granules against influenza A virus through regulating the inflammatory responses by TLR7/MyD88 signaling pathway. J Ethnopharmacol 2021; 275:114063. https://doi.org/10.1016/j.jep.2021.114063
15. Baley P. Acupuncture and Chinese herbal therapy for acute upper respiratory disease in a geriatric Quarter horse. Am J Trad Chin Vet Med 2009; 4(1):52-57. https://doi.org/10.59565/EDGH4562
16. Law A, Yang C, Lau A et al. Antiviral effect of forsythoside A from Forsythia suspensa (Thunb.) Vahl fruit against influenza A virus through reduction of viral M1 protein. J Ethnopharmacol 2017; 209:236-247. https://doi.org/10.1016/j.jep.2017.07.015
17. Feldman C, Anderson R. The role of co-infections and secondary infections in patients with COVID-19. Pneumonia 2021; 13(5): 1-15. https://doi.org/10.1186/s41479-021-00083-w