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

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ABSTRACT

A randomized controlled study was designed to investigate the efficacy of a combined treatment of the Chinese herbal medicine *Yin Qiao San* and antibiotic medicine (Group B; N = 32) for treating naturally occurring upper respiratory infections in shelter cats, where conventional antibiotic medicine alone was used as the control treatment (Group A; N = 19). A numerical clinical sign scoring system was devised with points given for conjunctivitis, sneezing, nasal discharge, lower respiratory signs, systemic signs and oral cavity involvement. Clinical sign scores for each cat were obtained before treatment and on days 2, 4, 6, 8 and 10 after initiation of treatment. The mean clinical scores of each group on days 2, 4, 6, 8 and 10 were compared to pre-treatment scores using the Wilcoxon signed rank test to determine the significance of clinical sign score reduction. There was no significant reduction in mean clinical sign scores for Group A on Days 2, 4 and 10, but a significant reduction occurred on Days 6 and 8 (p=0.002 and 0.0006, respectively). On the other hand, by Day 4 in Group B there was a significant improvement (p<0.001), and remained significant throughout Days 6, 8 and 10. No adverse effects occurred. The combination of *Yin Qiao* and Clavamox or doxycycline resulted in faster and more significant clinical improvement of cats with URI than antibiotics alone and was cost effective and had no adverse side effects.

Key words: Feline upper respiratory infection, herpes virus, calicivirus, *Yin Qiao San, Yin Chiao San*, Chinese herbal medicine, traditional Chinese veterinary medicine, cats

ABBREVIATIONS

FCV	Feline calicivirus
FHV-1	Feline herpes virus 1
IL-1 β	Interleukin 1 beta
IL-6	Interleukin 6
M±SD	Mean plus or minus the standard deviation
TCVM	Traditional Chinese veterinary medicine
TFF3 mRNA	Trefoil factor 3 messenger ribonucleic acid
TGF-β	Transforming growth factor beta
TNFα	Tumor necrosis factor alpha
URI	Upper respiratory infection(s)

Feline upper respiratory infections (URI) are extremely common in cats in animal shelters due to poor ventilation, stress-induced immunosuppression and overcrowded conditions.[1-3] Conjunctivitis, rhinitis and gingivostomatitis are obvious in some cats while others are asymptomatic carriers. Although caused by a variety of organisms, feline herpes virus 1 (FHV-1) and feline calicivirus (FCV) are the most common infectious agents.[1-3] *Mycoplasma felis, Bordetella bronchiseptica,* and *Chlamydophila felis* infections also occur.[4-5] The presence of these organisms can be confirmed by reverse transcription polymerase chain reaction (RT-PCR) assays.[5] Other secondary bacterial infections can further complicate the disease process. Conventional treatment often includes amoxicillin-clavulanic acid, cefovecin, doxycycline and fluroquinolones.[4,6] Antiviral treatments with mefloquine and lithium chloride have been recently evaluated *in-vitro* for FCV infections, but *in-vivo* studies are lacking.[7,8] Vaccination programs may reduce the severity or control the spread of infections, but many strains are vaccine resistant.[3] The result is that many cats are euthanized in order to control outbreaks or are unadoptable because of a severe URI. Although conventional treatments exist, the recovery is slow and other treatment options are needed to hasten recovery.

From a traditional Chinese veterinary medicine (TCVM) perspective upper respiratory infections may be

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associated with Wind-Heat, Wind-Cold and Damp Heat.[9] The External pathogens Wind and Cold invade the body through the nose causing sneezing and a serous nasal discharge. If *Wei Qi* and Lung *Qi* are weak, Wind-Cold transforms into Wind-Heat causing upper respiratory disease with fever and a thick, yellow nasal discharge. The pulses are strong and rapid reflecting Excess Heat and the tongue is red and dry. Feline URI is often associated with a Wind-Heat pattern.[9]

A variety of Chinese herbal medicines have been prescribed for the treatment of human URI.[10] After a 2014 systemic review and meta-analysis of 7 acceptable randomized controlled trials, it was concluded that there was evidence that Chinese herbal medicines had potential positive effects in the treatment of human URI.[10]

Yin Qiao San, a Chinese herbal medicine suggested to treat Wind-Heat URI, was first recorded in *Wen Bing Tiao Bian* (Detailed Analysis of Epidemic Febrile Disease) written by Wu Tang in 1798.[11] The formula is named for its 2 King herbs *Jin Yin Hua* (Lonicera) and *Lian Qiao* (Forsythia) (Table 1). The primary actions are to relieve exogenous pathogenic factors, clear Heat and eliminate toxins (Table 1). The authors (Hirsch and Xie) have successfully used *Yin Qiao San* to prevent and treat URI in horses, dogs and cats.[9,11] *Yin Qiao San* has also been successfully used to treat pneumonia and pleuropnemonia of cattle in China.[11] In a case report, acupuncture and a modified *Yin Qiao San*[a] (with *Ban Lan Gen* [Isatis] and *Lu Gen* [Phragmites] added and *Dan Dou Chi* [Soya] deleted) were used to successfully treat upper respiratory disease in a geriatric Quarter horse.[12] One author (Hirsch) has also used *Yin Qiao San* clinically along with conventional antibiotic therapy to treat feline URI in cats in animal shelters.[9] Although experiential evidence and case reports have been published, no randomized controlled clinical studies are currently published in English on the effects of *Yin Qiao San* for the treatment of URI in cats or other animals.[8,10]

The objective of this randomized controlled clinical study in shelter cats with naturally occurring URI was to demonstrate the effectiveness of *Yin Qiao San* when combining its use with conventional antibiotics. The hypothesis was that a combination of *Yin Qiao San*[b] and Clavamox[c] or doxycycline would result in faster and more significant clinical improvement of cats with URI than either antibiotic alone and have no adverse side effects.

MATERIALS AND METHODS

Cats for the study were recruited from 3 different animal shelters in the United States: Cape May Animal Shelter in Cape May County, New Jersey, the Ulster County Society for the Prevention of Cruelty to Animals, in Kingston, New York and the Atlantic County Animal Shelter, Pleasantville, New Jersey. All animals were handled and housed according to the guidelines for standards of care in animal shelters and participation in the study was on a volunteer basis.[13]

A clinical sign scoring system was devised based on the presence or absence and type of conjunctivitis, sneezing, nasal discharge, lower respiratory signs, systemic signs and oral cavity involvement as outlined in Table 2 and Figure 1. The scoring system was a modification of an earlier published scoring system for URI in cats.[4] The scores were recorded and treatments were administered by 2-4 technicians from each of the 3 participating agencies. All technicians underwent training to enhance the consistency of scoring. Technicians scoring were aware of whether the cat was in Group A or B as they also administered the treatments.

Inclusion criteria were cats showing 1 or more of the signs of URI; that is, a clinical sign score of 1 or above (Table 2). Cats were excluded from the study if they had no evidence of URI or were deemed unable to be safely handled to examine the oral cavity or administer medication. As a cat with URI was identified they were randomly assigned to one of the groups using a computerized random number generator[d]. Cats for which an even number was generated were placed in a control group (Group A) receiving antibiotics alone and cats with odd numbers were placed in a treatment group (Group B) receiving antibiotics and *Yin Qiao San*[b] combined. Antibiotics included amoxicillin-clavulanate (Clavamox[c]) liquid at a dose of 13.75 mg/kg body weight twice daily or doxycycline (compounded) at approximately 4.5 mg/kg

Table 1: Ingredients of the Chinese herbal medicine *Yin Qiao* and their actions[11]

Pin Yin Name	English Name	Amount %	Actions
Jin Yin Hua	Lonicera	17.9	Clears Heat and Toxins
Lian Qiao	Forsythia	17.9	Clears Heat and Toxins, reduces swelling
Niu Bang Zi	Articum	10.7	Disperses Wind-Heat in Lung Channel
Jie Geng	Platycodon	10.7	Opens Lung *Qi*, relieves cough, dissolves Phlegm
Bo He	Mentha	10.7	Disperses Wind and Heat
Dan Dou Chi	Soja	8.95	Relieves the Exterior
Gan Cao	Glycyrrhiza	8.95	Harmonizes the formula
Jing Jie	Schizonepeta	7.1	Opens the surface to release Exterior pathogens
Dan Zhu Ye	Lophatherum	7.1	Clears Heat, relieves restlessness, promotes diuresis

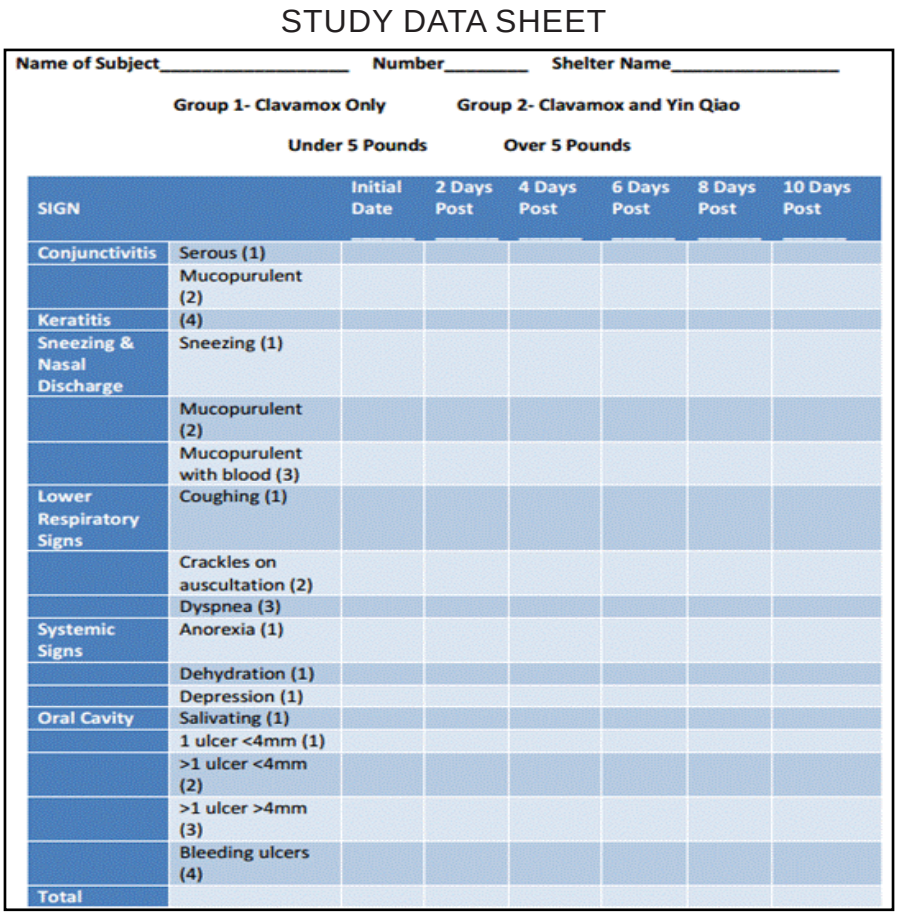
body weight once daily and was continued throughout the study. *Yin Qiao San*[b] tablets were administered twice daily at a dose of ½ tablet for cats under 5 pounds and 1 tablet for cats 5 pounds and over (approximately 0.13-0.25 g) twice daily and also continued throughout the study.

The daily clinical sign score for each cat was the sum

Table 2: Clinical scoring system for the evaluation of cats with upper respiratory infections

Anatomy	Clinical Signs		Points Assigned
Eyes	Conjunctivitis*	Serous conjunctivitis	1
		Mucopurulent conjunctivitis	2
	Keratitis		4
Nose	Sneezing		1
	Nasal discharge*	Mucopurulent only	2
		Mucopurulent with blood	3
Lungs	Lower respiratory*	Coughing	1
		Crackles on auscultation	2
		Dyspnea	3
Oral cavity	Salivation		1
	Oral ulcers*	1 ulcer <4mm	1
		Multiple ulcers <4mm	2
		Multiple ulcers >4mm	3
		Bleeding ulcers	4

If signs are not present a score of 0 is given; *select only one clinical sign (highest score)



STUDY DATA SHEET

Name of Subject_____ Number_____ Shelter Name_____

Group 1- Clavamox Only Group 2- Clavamox and Yin Qiao

Under 5 Pounds Over 5 Pounds

SIGN		Initial Date	2 Days Post	4 Days Post	6 Days Post	8 Days Post	10 Days Post
Conjunctivitis	Serous (1)						
	Mucopurulent (2)						
Keratitis	(4)						
Sneezing & Nasal Discharge	Sneezing (1)						
	Mucopurulent (2)						
	Mucopurulent with blood (3)						
Lower Respiratory Signs	Coughing (1)						
	Crackles on auscultation (2)						
	Dyspnea (3)						
Systemic Signs	Anorexia (1)						
	Dehydration (1)						
	Depression (1)						
Oral Cavity	Salivating (1)						
	1 ulcer <4mm (1)						
	>1 ulcer <4mm (2)						
	>1 ulcer >4mm (3)						
	Bleeding ulcers (4)						
Total							

Figure 1: Data sheet completed on each cat before treatment and on days 2, 4, 6, 8 and 10 after initiating treatment

of all the scores in each category on the scoring sheet for that day (Table 2, Figure 1). Clinical sign scores for each cat were obtained before treatment began and on days 2, 4, 6, 8 and 10 after the initiation of treatment. A mean score was calculated[e] for each evaluation day on all the cats in Group A and also all the cats in Group B. Due to the outcome data not being normally distributed (Shapiro-Wilk normality test; $p < 0.05$), Wilcoxon tests (nonparametric version of t test) were applied for mean score comparisons. The Group A and B pretreatment mean clinical scores were statistically compared using the Wilcoxon Rank Sum test[e] (nonparametric version of two-sample t test) to determine if there was a significant difference between the 2 groups prior to treatment. The mean clinical scores of Group A on days 2, 4, 6, 8 and 10 were compared to the pre-treatment (day 0) score for the group using the Wilcoxon Signed Rank test[e] (nonparametric version of paired t test) to determine significance of improvement within the group. The same was done for Group B. A p value less than (<) 0.05 was considered statistically significant.

RESULTS

Fifty cats met the inclusion criteria and became part of the study. There were fewer even numbers than odd numbers so there were 19 in the Group A (antibiotics alone) and 31 in Group B (antibiotics and *Yin Qiao San*). In Group A, 14 cats weighed less than or equal to (< or =) 5 pounds and 5 cats that weighed over 5 pounds. In Group B 18 cats weighed < or = 5 pounds and 13 cats weighed over 5 pounds. Being a multicenter study, 17 cats were located at Cape May Animal Shelter, 11 cats at the Ulster County Society for the Prevention of Cruelty to Animals and 22 cats at the Atlantic County Animal Shelter (Table 3). Gender, breed, neutered status and other physical traits (such as color) were not used as characteristic parameters. Also, duration in the shelter environment was not considered. In Group A 17/19 (89%) cats were administered Clavamox and 2/19 (10.5%) doxycycline. In Group B 21/31 (68%) cats were administered Clavamox and 10/31 (32%) doxycycline.

The individual and group mean clinical sign scores are listed in Tables 4 and 5 from Day 0 (prior to treatment)

Table 3: Number of cats in each treatment group enrolled at each shelter

	Cape May Animal Shelter	Ulster County Society for the Prevention of Cruelty to Animals	Atlantic County Animal Shelter	
Group A	9	4	6	**19**
Group B	8	7	16	**31**
	17	**11**	**22**	**50**

Table 4: Individual and group clinical scores at different time points in cats receiving antibiotics only (Group A)

Cat Number	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Shelter*
1	1	1	1	1	1	0	CM
2	2	1	1	1	0	0	CM
3	1	2	1	0	0	0	CM
4	2	3	0	0	1	4	UC
5	2	2	2	0	1	4	UC
6	2	1	1	0	0	0	UC
7	3	3	1	1	1	0	CM
8	7	8	7	6	1	1	CM
9	1	2	3	1	0	0	UC
10	3	1	1	1	0	1	CM
11	5	3	5	5	2	3	CM
12	5	5	5	4	4	7	AC
13	4	5	3	1	1	6	AC
14	2	3	4	2	2	2	AC
15	1	1	1	1	1	0	AC
16	1	1	1	1	0	0	AC
17	1	1	1	1	1	0	AC
18	2	1	2	1	1	0	CM
19	2	1	3	1	1	0	CM
M±SD	2.47±1.68	2.37±1.89	2.26±1.85	1.47±1.68	0.95±0.97	1.47±2.25	

M±SD = mean plus or minus the standard deviation; *CM = Cape May Animal Shelter in Cape May County, NJ; UC= Ulster County SPCA, in Kingston, NY; AC= Atlantic County Animal Shelter, in Pleasantville, NJ

and on Days 2, 4, 6, 8 and 10 after initiation of treatment. The clinical sign scores on initial inclusion prior to treatment ranged from 1-7 (M±SD = 2.47±1.68) for Group A, and 1-6 (M±SD = 2.77±1.31) for Group B. Although the mean score prior to treatment for Group A appeared to be less than Group B, when compared statistically, there was no significant difference (p= 0.1598). Side-by-side comparisons of the mean clinical sign scores between Group A and Group B at different time points are shown in Figure 2.

The results of the comparisons of mean clinical sign scores of Group A (antibiotics alone) on Day 0 and subsequent examination days are outlined in Table 6. There was no significant reduction in clinical sign scores for Group A on Days 2 and 4 (p=0.644 and 0.503, respectively), but significant reduction occurred on Days 6 and 8 (p=0.002 and 0.0006, respectively). However by day 10 the mean clinical sign score had increased again and was not significantly different than prior to treatment (p=0.082).

The results of the comparisons of mean clinical sign scores of Group B (antibiotics and *Yin Qiao San*) on Day 0 and subsequent examination days are outlined in Table 7. Similarly, on Day 2 the improvement was not significant (p = 0.609). But by Day 4 there was a significant improvement (p = 0.0008), and remained significant on Days 6, 8 and 10 (p = $7.9\times10^{-6,}$ 9.1×10^{-6}, and 0.0002, respectively). No adverse side effects were noted in cats receiving *Yin Qiao San* combined with Clavamox or doxycycline.

In both groups the mean clinical sign score on Day 10 increased when compared to Day 8 (Tables 4 and 5). This was because in Group A 6/19 (32%) cats (2 from each shelter) had increased clinical sign scores and Group B had 4/31 (13%) cats (1 or 2 from each shelter) had increased clinical scores (Tables 3 and 4). Although the mean clinical sign score of Group B was increased on Day 10, there was still a very significant improvement compared to the Day 0 scores. The worsening of 6 cats in Group A resulted in a loss of significant improvement when Day 10 was compared to Day 0 for the group.

Table 5: Individual and group clinical scores at different time points in cats receiving antibiotics and *Yin Qiao* san (Group B)

Cat Number	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Shelter
20	3	0	3	3	1	1	UC
21	5	5	4	1	1	0	UC
22	3	3	3	1	0	0	CM
23	2	2	0	2	1	2	UC
24	1	1	1	0	0	0	CM
25	6	5	4	4	1	1	CM
26	2	1	2	0	0	0	UC
27	2	2	1	0	0	1	UC
28	5	4	3	3	3	4	CM
29	5	5	4	4	4	4	CM
30	6	1	1	0	0	0	UC
31	3	5	4	2	2	2	UC
32	3	1	1	1	1	0	CM
33	3	3	3	3	3	6	AC
34	2	2	0	0	0	0	AC
35	2	3	4	2	2	1	AC
36	4	4	4	4	4	6	AC
37	2	2	2	2	2	1	AC
38	2	4	1	0	0	0	AC
39	2	4	1	0	0	0	AC
40	2	4	1	0	0	0	AC
41	2	2	1	1	1	1	AC
42	2	1	1	1	1	1	AC
43	2	2	2	1	2	2	AC
44	3	3	3	2	1	0	AC
45	2	2	1	1	1	1	AC
46	2	2	1	1	1	1	AC
47	2	2	1	1	1	1	AC
48	2	2	1	1	1	1	AC
49	2	1	1	1	2	2	CM
50	2	1	1	1	1	0	CM
M±SD	2.77±1.31	2.59±1.43	1.94±1.31	1.39±1.26	1.19±1.14	1.26±1.65	

M±SD = mean plus or minus the standard deviation; *CM = Cape May Animal Shelter in Cape May County, NJ; UC= Ulster County SPCA, in Kingston, NY; AC= Atlantic County Animal Shelter, in Pleasantville, NJ

In addition to the within-group (self-control) analysis for observing and comparing how quickly the improvement took place in each group, the study further conducted the between-group comparisons in mean improvement score on each of the 5 post-treatment examining days (Days 2, 4, 6, 8, and 10). For each of the 5 examining days, Group B had better mean improvement score than Group A. However, the results from the statistical tests (Wilcoxon Rank Sum test) did not support the statistical significance (with 0.05 significance level) of the greater improvements in Group B over Group A on any of the post-treatment examining days (p = 0.741, 0.058, 0.302, 0.819, and 0.540, respectively).

DISCUSSION

This is the first randomized controlled investigation of the effectiveness of using a Chinese herbal medicine combined with conventional antibiotics for the treatment

Table 6: Group A comparison of mean clinical scores on pretreatment Day 0 to Days 2, 4, 6, 8 and 10 after initiation of treatment to determine significant improvement

Days Compared	Pretreatment Score	Day Score	*p*-value	Significance
Day 0 to Day 2	2.47±1.68	2.37±1.89	0.644	Not significant
Day 0 to Day 4	2.47±1.68	2.26±1.85	0.503	Not significant
Day 0 to Day 6	2.47±1.68	1.47±1.68	0.002	Very significant
Day 0 to Day 8	2.47±1.68	0.95±0.97	0.0006	Very significant
Day 0 to Day 10	2.47±1.68	1.47±2.25	0.082	Not significant

$p<0.05$= Significant; $p<0.01$= Very significant

Table 7: Group B comparison of mean clinical scores on pretreatment Day 0 to Days 2, 4, 6, 8 and 10 after initiation of treatment to determine significant improvement

Days Compared	Pretreatment Score	Day Score	*p*-value	Significance
Day 0 to Day 2	2.77±1.31	2.59±1.43	0.609	Not significant
Day 0 to Day 4	2.77±1.31	1.94±1.31	0.0008	Very Significant
Day 0 to Day 6	2.77±1.31	1.39±1.26	7.9×10^{-6}	Very significant
Day 0 to Day8	2.77±1.31	1.19±1.14	9.1×10^{-6}	Very significant
Day 0 to Day 10	2.77±1.31	1.26±1.65	0.0002	Very significant

$p<0.05$= significant; $p<0.01$= very significant

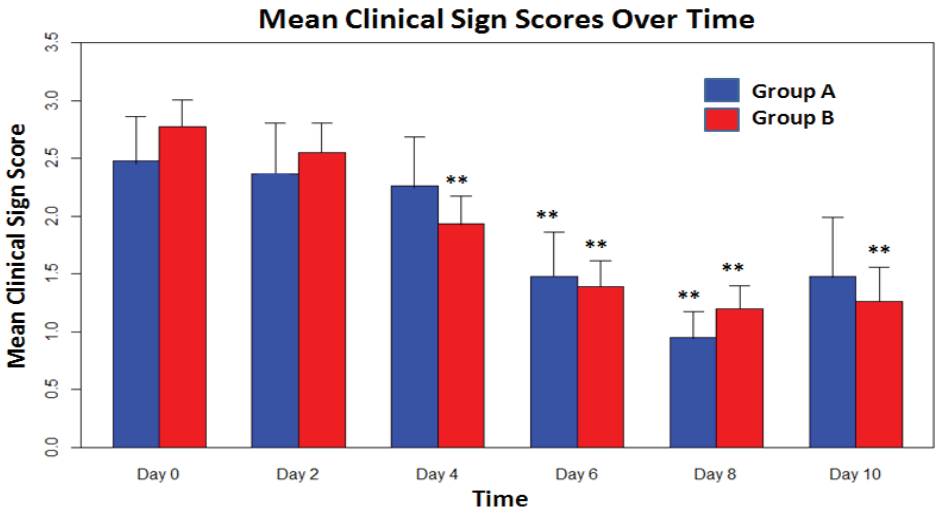


Figure 2: Changes in mean clinical sign scores from Day 0 prior to treatment and on Days 2, 4, 6, 8 and 10 after initiation of treatment; * significant reduction of clinical sign score $p<0.05$ as compared to pretreatment score for the group; ** very significant reduction of clinical sign score $p<0.01$ as compared to pretreatment score for the group; Group A (antibiotics alone) had a very significant reduction of clinical signs on Days 6 and 8, but not on any other days: Group B (antibiotics plus *Yin Qiao San*) started having a very significant reduction of clinical signs by Day 4 and remained very significant on Days 6, 8 and 10 of treatment.

of feline URI. The results of the study supported the hypothesis that a combination of *Yin Qiao San* and Clavamox or doxycycline would result in faster clinical improvement of cats with URI than either Clavamox or doxycycline alone and have no adverse side effects. By Day 4 cats treated with *Yin Qiao San* and antibiotics had significantly reduced clinical sign scores, and the improvement remained significant through Days 6, 8 and 10. On the other hand, the group treated with antibiotics alone showed significant improvement only after Day 6. This is especially important in a shelter situation, as affected cats are less likely to be euthanized because of their disease and become available for adoption faster.

On Day 10 of treatment, 6 cats in Group A and 4 cats in Group B had higher clinical sign scores than on Day 8 indicating worsening of clinical signs. Unable to control exposure to URI organisms from incoming cats, it was suspected that these cats contacted FCV in the shelter, which resulted in worsening of the URI. *Yin Qiao San* seems to have had a protective effect in Group B as only 13% of cats had increased clinical scores on Day 10 compared to 32% of cats in Group A. Since the cats in Group B on *Yin Qiao San* were doing so much better than Group A, the technicians later added *Yin Qiao San* to the treatment of several cats in Group A. Treatment on all cats was continued until the clinical signs resolved.

Experimental and clinical research provides some insights into the possible conventional mechanisms of action of *Yin Qiao San* on the respiratory system.[14-19] It is suspected that *Yin Qiao San* has protective effects on the respiratory system by improving the function of the respiratory mucosal immune system.[14] In an experimental murine study, a combined formula of *Xi Jiao Di Huang Tang* and *Yin Qiao San* inhibited the production of inflammatory cytokines and free radicals in mice with viral pneumonia caused by the influenza virus.[15] In another murine model of influenza viral pneumonia, *Yin Qiao San* reduced histological evidence of damage to lung tissue and reduced influenza virus hemagglutination titers compared to controls.[16]

In a human study of patients with dementia, the administration of either *Ma Xing Gan Shi Tang, Yin Qiao San* or *Xiao Qing Long Tang* reduced the number of patients developing pneumonia, a common complication for dementia patients, compared to those patients not receiving Chinese herbal medicine.[17] In another randomized study of humans with H1N1 influenza virus infections, treatment outcomes of 4 groups were compared: 1) combination of *Ma Xing Shi Gan* and *Yin Qiao San* alone, 2) the antiviral medication oseltamivir (Tamiflu[g]) alone, 3) a combination of *Ma Xing Shi Gan, Yin Qiao San* and oseltamivir and 4) a control group with no treatment.[18] Time to fever resolution was significantly less for the combination of *Ma Xing Shi Gan, Yin Qiao San* and oseltamivir compared to oseltamivir alone. In another study *Yin Qiao San* significantly reduced fever in children demonstrating its anti-pyretic effects.[19]

The anti-inflammatory and anti-viral effects of *Yin Qiao San* come primarily from 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).[11] In an *in-vivo* murine model of acute lung inflammation induced by lipopolysaccharide, *Jin Yin Hua* (Lonicera) increased the expression of interleukin 10 (IL-10), an important anti-inflammatory cytokine, which resulted in reduction of the proinflammatory cytokines tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1 β) and interleukin 6 (IL-6).[20] A reduction of proinflammatory cytokines was also found in an *in-vitro* study of the anti-inflammatory effects of flavonoids extracted from *Jin Yin Hua* (Lonicera) on microglia stimulated with lippolysaccharide.[21]

Anti-inflammatory mechanisms of Forsynthin, a major lignin found in *Lian Qiao* (Forsythia) has been studied in *in-vitro* and *in-vivo* murine models of viral and bacterial pneumonia, sepsis and cytokine-driven inflammation.[22] It was found that Forsynthin acts as an immunosuppressant by inhibiting phosphodiesterase 4 (PDE4) in inflammatory and immune cells. Further Forsythia has been shown to have direct effects on the influenza A viruses.[23] *Jin Yin Hua* (Lonicera), *Lian Qiao* (Forsythia) and *Jie Geng* (Platycodon) alone and combined were studied in another murine model of lipopolysaccharide induced lung inflammation.[24] Animals in the group that were administered a combination of all 3 herbs had reduced lung inflammation on histopathological examination compared to the untreated control group. Significantly or very significantly reduced ($p<0.05$ or $p<0.01$) transforming growth factor beta (TGF-β), released from macrophages following inflammatory stimuli, as well as reduced IL-1β and WBC counts were found in bronchial alveolar lavage fluid in the herbal treated group. Further, trefoil factor 3 messenger ribonucleic acid (TFF3 mRNA), which protects respiratory mucosa, was very significantly increased ($p<0.01$) in lung tissue from the group administered all 3 herbs. The conclusion was *Jie Geng* (Platycodon) acts synergistically with the King herbs found in *Yin Qiao San* to further reduce inflammatory cytokines and protect lung tissue.[24] The anti-viral activity of aqueous extracts of *Bo He* (Mentha) against Influenza A, Newcastle disease virus, herpes simplex virus and vaccinia virus have been shown since the 1960s and the anti-inflammatory, antibacterial and anti-viral effects has been extensively studied *in-vitro* and *in-vivo* since that time.[25] *Niu Bang Zi* (Articum) another major component of *Yin Qiao San* has been shown to have anti-inflammatory, anti-oxidant, anti-bacterial and antifungal effects in many *in-vitro* and *in-vivo* studies.[26,27]

Limitations to the study included housing cats in a shelter facility and statistical inter- versues intra-group comparison. The ideal clinical study situation would have been 50 cats housed in 1 location, isolated from other

incoming cats, living under the same conditions, treated with the same antibiotic and evaluated by one veterinarian or well trained technician that was blinded to the treatment group. In reality the variables in clinical studies cannot always be that well controlled. The uncontrollable variables in the current multicenter animal shelter clinical study included: 1) varying administrative organizations, policies and commitment to the study, 2) use of different antibiotics by different shelters, 3) differences in interpretation of clinical signs by multiple technicians, 4) technician bias because they were unblinded to the group assignment and 5) exposure of cats to viruses and secondary bacterial infections associated with the large turnover of cats in a shelter environment.

During the study design, it was determined that a sample size of 25 subjects in each group (50 total) was sufficient to ensure at least 90% test power (for both paired-t tests and nonparametric Wilcoxon Sign Rank tests for each within-group analysis) to detect a difference of one sample standard deviation with a 0.05 significance level. Such a sample size would also give 89% power (for both two sample t tests and nonparametric Wilcoxon Rank Sum tests for between-group comparison) to detect a difference of one (pooled) sample standard deviation with a 0.05 significance level. In order to get 50 cats for the study within the time frame, involvement of 3 different animal shelters was necessary. Two shelters exclusively used Clavamox for feline URI, but would switch to doxycycline if suspected FCV outbreaks occurred. The other shelter used either Clavamox or doxycycline. Due to work schedules and number of available technicians at any given time, more than 1 technician at each of the 3 locations completed the clinical sign scoring sheet on the predetermined days. Although training sessions for technicians were provided, interpretation of clinical signs most likely varied slightly among individuals. In addition, due to limited technician availability, the study was conducted unblinded, with a technician scoring and treating the same cat. Without being blinded there could have been positive or negative bias that affected the interpretation by the technician based on a preconceived notion of whether Chinese herbal medicine would be helpful or not. Even with all these study challenges, the differences in clinical scores before treatment compared to days 6, 8 and 10 in the 31 cats treated with antibiotics and *Yin Qiao San* were very significant ($p<0.01$). The difference in the groups was so clear to those working with them that eventually many cats were given *Yin Qiao San* along with antibiotics until the URI had resolved.

With all these challenges, statistically valid inter-group comparison was difficult to demonstrate versus intra-group comparison of animals with their own pretreatment baseline. Based on the results from the first (within-group) analysis, it would be anticipated that only on Day 4 the mean inter-group improvement score in Group B was significantly greater than that in Group A. This hypothesis was supported marginally ($p = 0.058$) by this between-group comparison. A study with a larger number of animals (and more balanced groups) in facilities with better quarantine procedures could provide more statistical power to claim this significance.

In summary it was found that for naturally occurring URI of cats, a combination of *Yin Qiao San* and either Clavamox or doxycycline resulted in faster resolution of clinical signs than with antibiotics alone. There were no adverse side effects and the *Yin Qiao San* tablets were relatively easy to administer. *Yin Qiao San* costs 6-8 cents/tablet and with the usual dose of 0.5-1 tablet twice daily, based on their weight, *Yin Qiao San* was also cost effective. Discounted Clavamox in the shelter situation is usually about $3/day/cat and compounded doxycycline liquid required for feline administration can cost even more depending on the source of the drug. Clinicians should be aware that some human over-the counter *Yin Qiao San* preparations may also contain acetaminophen, which causes feline liver and red blood cell damage resulting in mortality in some animals. Also TCVM clinicians should be alert to modifications in the original *Yin Qiao San* formulations with the addition and deletion of other herbal ingredients. Based on the results of this study *Yin Qiao San,* along with Clavamox or doxycycline is recommended for the treatment of feline URI. In the future, blinded, prospective studies are needed to compare antibiotics alone versus *Yin Qiao San* administered either alone or combined with antibiotics to determine the optimum treatment of feline URI.

FOOTNOTES

a. Jing Tang Herbal, Reddick FL; www.tcvmherbal.com
b. *Yin Chiao Chieh Tu Pien* (extra concentrated tablets) by Plum Flower Brand, Mayway, San Francisco CA
c. Clavamox, Zoeitis Corporation, Kalamazoo, MI
d. https://www.easycalculation.com/statistics/standard-deviation.php
e. The R Foundation for Statistical Computing; R version 3.2.2
f. Roche Pharmaceuticals, Basel, Switzerland;, http://www.roche.com/

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