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Clinical Studies

Canine Parvoviral Enteritis Mortality in Shelter Dogs Receiving Conventional Medical Therapy Compared to Integrative Treatment with the Chinese Herbal Medicine, *Zhi Li Tang*: A Pilot Study

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ABSTRACT

Canine parvoviral (CPV) enteritis is a contagious infection, which particularly affects young dogs, and is associated with high morbidity and mortality rates in the canine population. This study sought to determine whether including the Chinese herbal medicine formula, *Zhi Li Tang* (ZLT, Red Back Door), in conjunction with conventional therapy can decrease mortality of shelter dogs with CPV enteritis. Nineteen canine subjects from a shelter population diagnosed with CPV enteritis were randomly assigned to a Control Group which received conventional supportive treatment, or an Integrative Treatment Group which received ZLT along with conventional supportive treatment. Study duration was 14 days unless the subject died, or all clinical signs associated with CPV enteritis had resolved. The outcome measurement was a binary response of mortality within the 14-day trial. In the Control Group, there was a 43% (3/7) mortality rate compared to 0% mortality in the Integrative Treatment Group (12/12, all dogs recovered) within the 14-day study period. All subjects that survived in both study groups had resolution of CPV clinical signs by the end of the study. Despite the small sample size in this pilot study, there was a statistically significant (p=0.036) improvement in survival for the dogs receiving *Zhi Li Tang* in addition to conventional therapy when compared to the dogs receiving conventional therapy only. The results from this study suggest integrating ZLT with conventional treatment of CPV enteritis could reduce mortality from CPV infections in shelter dogs. Further investigation of these findings with larger studies is needed to confirm study results.

Keywords: canine, Chinese herbal medicine, parvoviral enteritis, Red Back Door, *Zhi Li Tang*

ABBREVIATIONS: CPV: canine parvoviral; IV: intravenous; MR: mortality rate; SQ: subcutaneous; TCVM: traditional Chinese veterinary medicine; ZLT: *Zhi Li Tang*

Canine parvoviral (CPV) enteritis is an infectious disease that can spread to dogs of any age, with puppies being the most susceptible. It is highly contagious, usually spread by fecal-oral route and is associated with high mortality rates.[1] Unlike most other viruses, CPV is very stable in the environment and can survive for more than a year in contaminated feces, vomitus or fomites. It is resistant to heat, detergents, alcohol, and most disinfectants (requires 1:30 bleach solution), making it a very important pathogen in highly populated areas such as shelters, pet stores, and kennels.[2-3] Without treatment, mortality rates of CPV enteritis infection in dogs have been reported to be as high as 90.9%.[4]

Virus destruction of rapidly dividing cells, such as crypt intestinal epithelial cells, lymph nodes, and bone marrow precursor cells results in villous atrophy, intestinal mucosal barrier destruction, malabsorption and profound leukopenia. This pathology leads to the clinical signs of profuse hemorrhagic diarrhea, vomiting, dehydration/ hypovolemia, metabolic acidosis (or alkalosis), bacterial translocation with coliform septicemia/endotoxemia, multiorgan dysfunction and death.[3] Comorbid stressors (e.g. parasites, crowding, weaning, unsanitary conditions) may exacerbate the disease.[3]

There is no known single effective treatment for CPV enteritis currently. Conventional treatment is largely supportive and includes intensive hospitalization with

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intravenous (IV) fluid therapy, correction of hypoglycemia/ electrolyte disturbances, antibiotics, antiemetics, and preventing hypothermia. Antibiotic use is recommended due to disruption of the intestinal lining, creating concern for sepsis from translocation of bacteria such as *Escherichia coli* and *Clostridium perfringens*.[1] Common antibiotics used include cefovecin, amoxicillin, metronidazole, enrofloxacin, and sometimes an aminoglycoside.[1,3] Reported survival rates for dogs with CPV enteritis treated with antibiotics and supportive care vary widely from 64-92%.[5]

Shortcomings of current treatments include cost as well as the intensity and duration of treatments needed. The average hospitalization time reported in 1 study was 4.6 days.[6] Depending on the type and location of a hospital, this could cost approximately US$1,500 to $3,000; which even with the high cost does not guarantee survival of the dog. At the shelter and rescue level, due to the high risk of spreading infection, unduly expensive treatment costs, and lack of success; many shelters and rescues often elect to euthanize the affected dogs instead of pursuing treatment. A therapy that could shorten hospitalization, decrease cost of treatment, and increase survival rate is currently needed for the treatment of CPV enteritis.

A veterinary specific, patented Chinese herbal medicine, *Zhi Li Tang* (ZLT, Red Back Door), was first introduced in 1994. The herbal medicine formula was specifically designed for treatment of dogs infected with canine parvoviral infection demonstrating clinical signs of diarrhea with blood/mucus, vomiting, loss of appetite, and inflammation of the small intestine.[7] Its ingredients consist of Phellodendron (*Huang Bai*), Coptis (*Huang Lian*), Scutellaria (*Huang Qin*), Gardenia (*Zhi Zi*), Sanguisorba (*Di Yu*), Pinellia (*Ban Xia*), Atractylodes (*Cang Zhu*), Angelica (*Dang Gui*), Paeonia (*Bai Shao Yao*), Astragalus (*Huang Qi*), Dioscorea (*Shan Yao*), Saussurea (*Mu Xiang*), Aurantium (*Zhi Ke*), Bambusa (*Zhu Ru*), and Licorice (*Gan Cao*) (Table 1).[8] In traditional Chinese veterinary medicine (TCVM), its actions are to clear Damp-Heat, move *Qi* to relieve pain, and tonify Spleen *Qi* to stop diarrhea. Indications for use include: treatment of Damp-Heat in the gastrointestinal tract with Spleen *Qi* Deficiency, diarrhea containing blood/mucous, vomiting, anorexia accompanied by TCVM clinical signs of a red tongue and fast or weak pulse.[9] There is no known contraindication for using this herbal formulation with the direction to use up to 3 weeks, and to stop administration as soon as diarrhea resolves.[7]

Even though no specific study could be found regarding ZLT's efficacy against CPV enteritis, various antiviral, antibacterial, and pain-alleviating effects have been reported with many of its components and their constituents. Phellodendron, Coptis, Scutellaria, Gardenia, Sanguisorba, Atractylodes, Angelica, Paeonia, Saussurea and Chinese Licorice have both antiviral and anti-bacterial effects.[10-28] Pinellia, Paeonia, Astragalus, Dioscorea, and Aurantium have antibacterial activity.[29-39] All herbs in this Chinese medicine formula with the exception of Phellodendron and Pinellia help alleviate pain (Table 2).[18,40-51]

Table 1: Ingredients of the Chinese herbal medicine *Zhi Li Tang* (Red Back Door[a]) and their TCVM actions

***Pin Yin* Name**	**English Common Name**	**TCVM Actions**
Huang Bai	Phellodendron	Clears Heat-toxin, detoxifies
Huang Lian	Coptis	Clears Damp-Heat, detoxifies
Huang Qin	Scutellaria	Clears Damp-Heat, detoxifies
Zhi Zi	Gardenia	Clears Damp-Heat, detoxifies
Di Yu	Sanguisorba	Cools Blood, stops bleeding
Ban Xia	Pinellia	Transforms Phlegm, stops vomiting
Cang Zhu	Atractylodes	Dries up Damp, strengthens Spleen
Dang Gui	Angelica	Nourishes Blood
Bai Shao Yao	Paeonia	Nourishes Blood
Huang Qi	Astragalus	Tonifies *Qi*
Shan Yao	Dioscorea	Tonifies *Qi*
Mu Xiang	Saussurea	Moves *Qi*, resolves pain
Zhi Ke	Aurantium	Moves *Qi*, resolves pain
Zhu Ru	Bambusa	Transforms Phlegm, stops vomiting
Gan Gao	Chinese Licorice	Harmonizes

Table 2: Summary of literature references for antiviral, antibacterial and/or analgesic effects of the constituent herbs that make up the Red Back Door Chinese herbal medicine formula (*Zhi Li Tang*).

Common Name (Chinese *Pin Yin*)	**Antiviral (References)**	**Antibacterial (References)**	**Analgesic (References)**
Phellodendron (*Huang Bai*)	7, 8	26	–
Coptis (*Huang Lian*)	7, 9	27	37
Scutellaria (*Huang Qin*)	10, 11	11	38
Gardenia (*Zhi Zi*)	12, 13, 14	28	39
Sanguisorba (*Di Yu*)	15, 16	15	15
Atractylodes (*Cang Zhu*)	17, 18, 19	30	40
Angelica (*Dang Gui*)	20, 21	31	41
Paeonia (*Bai Shao Yao*)	22	32	42
Dioscorea (*Shan Yao*)	23	34	44
Saussurea (*Mu Xiang*)	24	35	45
Chinese Licorice (*Gan Cao*)	25	25	48
Pinellia (*Ban Xia*)	–	29	–
Astragalus (*Huang Qi*)	–	33	43
Aurantium (*Zhi Ke*)	–	36	46
Banbusa (*Zhu Ru*)	–	–	47

As there have not been reports of scientific study on the efficacy of ZLT in the treatment of CPV enteritis in dogs, the objective of this pilot study was to determine whether using ZLT in conjunction with conventional therapy can reduce mortality in shelter dogs with CPV enteritis. The study hypothesized that subjects with CPV enteritis treated with the proposed integrative therapy (ZLT + conventional treatment) would have a lower mortality rate than conventional treatment only.

MATERIALS AND METHODS

Animals

The study was conducted at the KC Pet Project, an animal shelter located in Kansas City, Missouri, USA, where the shelter director and staff veterinarians provided informed consent for their animals to participate in the study. The subject population under investigation consisted of shelter dogs diagnosed with CPV enteritis. The dogs were either previously housed in the shelter and contracted CPV or were relinquished to the shelter due to CPV infection. The shelter director and all shelter staff members, who were not blinded to study group, were adequately trained to follow the study protocols, testing procedures, and medication administration. The enrollment inclusion criteria included: (1) at least 6 weeks old; (2) positive Idexx Parvo Snap test; and (3) deworming treatment with fenbendazole[a] (40 mg/kg) once daily for 5 consecutive days. Subjects with the following conditions were excluded: (1) additional clinical signs not associated with the gastrointestinal tract (e.g. seizures, respiratory disease, pneumonia); (2) moribund clinical condition at study start (Day 0); and (3) positive fecal floatation test for parasites not treatable by fenbendazole (i.e. coccidia).

Study Design

This was a 14-day randomized, controlled, open label pilot study. Each enrolled subject was randomly assigned using a random number generated from a statistical software program[b] into the Control Group or the Integrative Treatment Group. Both study groups received conventional supportive treatment for parvoviral enteritis including subcutaneous (SQ) or intravenous (IV) fluids based on dehydration level, antibiotic(s)[c-e], antiemetic[f], anti-diarrheal medication[g], dewormer[a] (start Day 0 through Day 4), and feeding every 12 hours (Table 3). Oral force-feeding was performed if a patient was not eating on its own. The duration of therapy continued for 14 days unless the patient died or recovered from the disease.

Table 3: Conventional supportive treatment received by both study groups to treat parvoviral enteritis during the 14-day clinical study

Treatment	Medication	Route	Dose
Fluids	Lactated Ringer Solution	SQ or IV	Based on dehydration level
Antibiotic Selection	Ampicillin/Sulbactam[c]	Oral	15 mg/kg every 12 hours
	Ampicillin[d]	Oral	20 mg/kg every 12 hours
	Enrofloxacin[e]	SQ or Oral	2.5 mg/kg every 24 hours SQ or Oral
Antiemetic	Maropitant[f]	SQ or Oral	1 mg/kg every 24 hours
Antidiarrheal	Propectalin Paste[g]	Oral	1.0 ml/10 lb or 1 ml/4.5 kg every 8 hours
Dewormer	Fenbendazole[a]	Oral	50 mg/kg every 24 hours, 5 consecutive days

SQ=subcutaneous, IV=intravenous

Table 4: *Zhi Li Tang (*Red Back Door) rectal enema dosing chart. During study treatment, the herbal formula was given as an enema at 1gram per 11-20 pounds or 1 gram per 5.0-9.1 kg body weight. The goal for enema dosage (0.11 g/kg - 0.2 g/kg) was twice the amount recommended for oral route (0.1 g/kg) twice daily.

Body Weight (lb) (Dose: 1 g / 11-20 lb)	Body Weight (kg) (Dose: 1 g / 5.0-9.1 kg)	Dose (tsp)	Dose (mg)
Up to 5	Up to 2.27	1/8	250
6-10	2.72-4.55	1/4	500
11-20	5.0-9.1	1/2	1,000
21-30	9.55-13.64	3/4	1,500
31-40	14.09-18.18	1	2,000
41-50	18.64-22.73	1¼	2,500
51-60	23.18-27.27	1½	3,000

lb=weight in pound; kg=weight in kilogram; tsp=teaspoon; mg=milligram

In addition to conventional supportive therapy, the Integrative Treatment Group was dosed with the Chinese herbal medicine formula, ZLT, which was given rectally as a slurry at a dose based on the subject's body weight (1.0 g per 11-20 lb or 0.11 g/kg-0.2 g/kg) twice daily (Table 4). The formula was diluted with warm water (approximately 100°F or 38°C) and given as an enema, using a catheter tube[h] (8-10 French red rubber catheter) based on the patient's size, with the tube length trimmed to approximately 11 inches for easy application. It was retained rectally for 10 minutes for absorption by placing a towel or cotton balls at the anus to prevent leakage. The Control Group did not receive an enema treatment.

All treatments were administered during or immediately following feeding (whether force-fed or eating voluntarily) by trained personnel assigned to the parvovirus isolation ward for the day. These same individuals documented the energy level, fecal score, presence or absence of appetite

and vomiting, twice daily (morning and late afternoon). Additionally, they recorded the administration of ZLT and any other injectable or oral medications, gave IV or SQ fluids, and fed the patients. All conventional supportive therapies were continued until 24 hours past resolution of vomiting, diarrhea, lethargy, and anorexia. The ZLT treatment was continued for 2 doses after resolution of diarrhea or 28 doses, whichever came first. Any unusual behavior and/or adverse side effects from ZLT administration were to be recorded. Time and date for the deceased were recorded. All patients were kept in the parvovirus isolation ward for the 14-day quarantine period.

Data Analysis

Signalment data was documented for each study subject along with collection of the primary outcome data which was recorded as a binary outcome: "Yes" if the subject was deceased within the 14-day study period and as "No" otherwise. Secondary data, for those who recovered from CPV enteritis, was documentation of their recovery time. The patient was classified as having recovered from CPV enteritis when, within 14 days after starting the assigned treatments, all of the following clinical signs were resolved for more than 24 hours: (a) vomiting defined as producing vomitus; (b) lethargy defined as having an attitude score less than bright, alert, and responsive (BAR); (c) diarrhea defined as soft stool with or without shape (Purina Fecal Scale score 4 and above); (d) hematochezia defined as having blood in the stool whether diarrhea was present or not and recorded with each stool quality score; and (e) anorexia defined as not voluntarily eating in the last 24 hours.

The study hypothesized that the mortality rate (MR) of the Integrative Treatment Group population (MR_T) would be lower than that of the Control Group population (MR_C). Hence the study tested the statistical null hypothesis H_0: $MR_T = MR_C$ vs. the alternative H_A: $MR_T < MR_C$. 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.

A sample size of 12 subjects per group was planned for testing the hypothesis with Fisher's Exact test as described above, to ensure a 90% power to reject the H_0 with a 0.05 significance level, assuming MR_T=20% and MR_C=80%. All data graphic presentations and statistical analysis were performed using a commercial statistical software[b].

Table 5: Subject signalment data (mean±SD) summary statistics

	Control Group	Integrative Treatment Group	*p*-value
Age (months)	3.13±1.59	3.32±2.68	0.96
Weight (kg)	7.95±5.18	6.31±4.36	0.52
Sex (% female)	28.6%	66.7%	0.17

RESULTS

The study enrolled a total of 19 subjects that met the inclusion and exclusion criteria. An infectious respiratory disease outbreak at the shelter resulted in removal of some enrolled randomized study group dogs before Day 0 (study start). This resulted in 12 dogs assigned to the Integrative Treatment Group and 7 to the Control Group giving a total of 19 dogs on Day 0 of the study. This was less than the goal of 24 subjects (12 per group) initially calculated to give a 90% power to reject the H_0 (0.05 significance level) and reduced power to approximately 74% for comparing mortality rate, under the same statistical considerations and assumptions.

The subject signalment data, including age, weight and sex proportion, were compared between groups to confirm the group comparability for treatment outcome comparisons. No significant group difference was found (Table 5). There were a variety of breeds in each study group with most animals belonging to a mixed-breed category. In the Control Group there were 3 Labrador Retriever mixes, 2 terrier mixes, 1 Labrador Retriever, and 1 German Shepherd. In the Integrative Treatment Group, there were 2 terrier mixes, 3 Pitbull mixes, 2 Labrador Retriever mixes, 3 Rottweiler mixes, 1 Pitbull, and 1 mixed-breed dog.

All study dogs had at least one common clinical sign of CPV enteritis (vomiting, diarrhea, hematochezia, anorexia, lethargy) at the beginning of the study (Table 6). Of the 19 study subjects, 19/19 (100%) had diarrhea (7/7 control, 12/12 integrative), 17 (89%) had blood in their stool (6/7 control, 11/12 integrative), 16 (84%) had anorexia (6/7 control, 10/12 integrative), 15 (79%) had lethargy (7/7 control, 8/12 integrative), and 13 (68%) had vomiting (6/7 controls, 7/12 integrative).

Table 6: Summary of presenting clinical signs at study start (Day 0) for each dog according to group; with recovery (no evidence of a clinical sign for 24 hours) on Day 14 of each presenting sign highlighted in bold text

Control Group		Integrative Treatment Group	
Subjects	**Clinical Signs**	**Subjects**	**Clinical Signs**
C-1	**D, A, L**	T-1	**V, D, H, A, L**
C-2	V, D, H, A, L	T-2	**V, D, H, A, L**
C-3	V, D, H, A, L	T-3	**V, D, H, A, L**
C-4	**V, D, H, L**	T-4	**V, D, H, A, L**
C-5	**V, D, H, A, L**	T-5	**V, D, H, A, L**
C-6	**V, D, H, A, L**	T-6	**V, D, H, A, L**
C-7	V, D, H, A, L	T-7	**D, H, A, L**
N/A	N/A	T-8	**D, H, A, L**
N/A	N/A	T-9	**D, H, A**
N/A	N/A	T-10	**V, D, H**
N/A	N/A	T-11	**D**
N/A	N/A	T-12	**D, H, A**

V = vomiting; D = diarrhea; H = hematochezia; A = anorexia; L = lethargy; N/A = not available

There were no adverse effects associated with the administration of ZLT to patients or any conventional study treatment. All integrative treatment subjects survived the 14-day study period (12/12, 100%) giving a 0% mortality rate in this group. In the control dogs, 3 out of 7 subjects (3/7 = 43%) died within the 14-day timeline (Table 7). The mortality rate in the Integrative Treatment Group (0%) was significantly lower (p=0.036) than the Control Group (43%). Recovery time was defined as cessation of all documented common CPV enteritis clinical signs for at least 24 hours (vomiting, diarrhea, hematochezia, anorexia, lethargy). Among the 12 subjects in the Integrative Treatment Group, the mean±SD recovery time was 4.29±2.29 days. The recovery time in the controls that survived (4/7) was similar at 4.25±2.40 (Table 8). The difference between the two groups was not statistically significant (p=0.844).

Table 7: Mortality rate by Day 14 for each study group

	Control Group Subjects	Integrative Treatment Group Subjects	*p*-value
Mortality Rate	3/7 = 43%	0/12 = 0%	0.036*

*Fisher's Exact test, statistically significant $p<0.05$

Table 8: Recovery time (in days) for each individual subject

Control Group		Integrative Treatment Group	
Subjects	**CPV Recovery Time (Days)**	**Subjects**	**CPV Recovery Time (Days)**
C-1	4.5	T-1	5.5
C-2	Deceased	T-2	5.0
C-3	Deceased	T-3	5.5
C-4	3.0	T-4	3.5
C-5	7.5	T-5	3.0
C-6	2.0	T-6	2.0
C-7	Deceased	T-7	9.5
N/A	N/A	T-8	3.5
N/A	N/A	T-9	4.5
N/A	N/A	T-10	3.0
N/A	N/A	T-11	0.5
N/A	N/A	T-12	6.0
Mean±SD	**4.25±2.40**	**Mean±SD**	**4.29±2.29**

CPV = canine parvovirus enteritis; N/A = not available

DISCUSSION

Canine parvovirus is a highly contagious virus with significant morbidity and mortality in dogs under 6 months old. Despite the availability of vaccination programs for privately owned animals, shelters work with a steady influx of unvaccinated populations. The disease can create devastating outbreaks, even in the best equipped shelters, and is usually accompanied by poor treatment results causing many shelters to elect euthanasia of infected dogs versus treatment of the animals. The objective of this randomized, controlled open label clinical study was to determine whether integrating the Chinese herbal medicine, *Zhi Li Tang*, as an adjunct to conventional therapy would decrease mortality in shelter dogs infected with CPV enteritis. Study results found there was a 43% (3/7) mortality rate in the conventional treatment only dogs (controls) compared to 0% mortality rate (12/12 dogs recovered) in ZLT plus conventional treatment patients within the 14-day study period. All subjects that survived in both study groups had resolution of CPV clinical signs by the end of the study. Despite the small sample size in this pilot study, there was a statistically significant (p=0.036) improvement in survival for the dogs receiving *Zhi Li Tang* in addition to conventional therapy when compared to the dogs receiving conventional therapy only. These findings satisfied the study hypothesis of decreased mortality in shelter dogs treated with Chinese herbal medicine therapy as an adjunct to conventional treatment.

All study dogs had at least one common clinical sign of CPV enteritis (vomiting, diarrhea, hematochezia, anorexia, lethargy) at the beginning of the study, with most of the dogs having more than 1 of these clinical signs. Diarrhea was the most common presenting clinical sign and was present in all 19/19 (100%) study dogs. Blood in the stool, whether the fecal score was over 4 or not [2 subjects had a normal stool (fecal score of 3) but with fresh blood present], was the second most common finding and was present in 17/19 (89%) study dogs (6/7 control, 11/12 integrative). This was followed by 16/19 (84%) dogs with anorexia (6/7 control, 10/12 integrative), 15/19 (79%) with lethargy (7/7 control, 8/12 integrative), and 13/19 (68%) had vomiting at initial presentation (6/7 controls, 7/12 integrative). Other studies have noted lethargy and anorexia as the most common presenting clinical sign and then similar to this study, was followed by diarrhea and then vomiting.[3] The difference in initial presentation between this study and the literature reference may be due to earlier presentation of affected dogs in private practice versus later evaluation of dogs relinquished to a shelter. Dogs who survived the 14-day study had all clinical signs completely resolved by the end of the study. This was true for both the Control Group and Integrative Treatment Group. Among the 12 subjects in the Integrative Treatment Group, the mean±SD recovery time was 4.29±2.29 days. The recovery time in the controls that survived (4/7) was similar at 4.25±2.40. The difference between the two groups was not statistically significant (p = 0.844).

There are two studies using herbal medicine in the treatment of CPV enteritis that are most comparable to the present study. One study used an extract of *Saruma henryi Oliv.* (plant endemic to China) in experimentally infected puppies with CPV enteritis, and the other study by Yang et al. looked at three different herbal medicine formulations

for the treatment of dogs with CPV enteritis.[52,53] In the *Saruma henryi Oliv.* extract study, CPV dogs (n = 15) receiving Chinese herbal medicine along with conventional therapy, similar to the integrative group in the present study, had only 1 death (i.e. 7% mortality rate) over a 15-day trial period. The conventional treatment only dogs (n=10) in the study, in contrast had much higher mortality at 50% of the group, again similar to the present study.[52] The second study compared three different Chinese herbal medicine formulations as an adjunct to conventional supportive care (included IV fluids) in 205 dogs naturally infected with CPV.[53] The mortality rates for the three groups treated with different Chinese herbal medicine formulations were 4%, 21% and 12%; all significantly smaller than mortality in the control group. The composition of the most effective formula (mortality = 4%), which has similarities to ZLT, included Coptis (*Huang Lian*) 15g, Scutellaria (*Huang Qin*) 15g, Pueraria (*Ge Gen*) 8g, Poria (*Fu Ling*) 10g, Plantago (*Che Qian Zi*) 6g, Sanguisorba (*Di Yu*) 8g, Magnolia (*Hou Po*) 8g, Pogostemon (*Huo Xiang*) 6g, Corydalis (*Zi Jin*) 5g, Astragalus (*Huang Qi*) 12g, and Glycyrrhiza (*Gan Cao*) 8g. Comparing data from the two studies, ZLT and the formulation used in Yang et al.'s study have comparable treatment efficacy for CPV enteritis, with ZLT seemingly having slightly superior effects. One point of interest is that there were a number of herbs that were both in ZLT and the most successful formulation in Yang et al.'s study. These herbs include Coptis (*Huang Lian*), Scutellaria (*Huang Qin*), Sanguisorba (*Di Yu*), Astragalus (*Huang Qi*), and Glycyrrhiza (*Gan Cao*).

In an interesting detailed review of Chinese herbs used to treat CPV, Qiu et al. provide insights on Chinese herbal medicine formulas (3 classic and 4 novel prescriptions) that have been considered to have potential for treating CPV, as well as on single Chinese herbs and herbal extracts with anti-CPV activities.[54]

Plants are a rich source of pharmacologically active compounds, many of which have demonstrated antiviral activity.[55] Eleven of the constituent herbs of ZLT have demonstrated antiviral activity in investigational studies with molecular mechanisms of action defined in some of these. Coptis (*Huang Lian*) contains the plant isoquinoline alkaloid, berberine, which has demonstrated strong antiviral activity.[55] Recent studies have demonstrated the ability of this alkaloid to inhibit viral entry and replication through regulation of signaling pathways necessary for viral replication, and insertion into DNA which inhibits synthesis and reverse transcriptase activity. Berberine also affects host immune response leading to increased viral clearance.[55] Scutellaria (*Huang Qin*) has raised interest as a potential candidate for the development of a novel broad-spectrum antiviral drug. It contains a bioactive flavonoid glycoside, baicalin, whose antiviral molecular mechanisms of action have been widely investigated. Its antiviral activity includes: inhibition or stimulation of signaling pathways (e.g. JAK/STAT, NF-κB, toll-like receptor), optimizing interferons, T-cell subsets effects which improve antiviral immune function, and inhibition of cell apoptosis caused by virus infection.[56] Sanguisorba (*Di Yu*) has been used traditionally for infectious diseases. It contains a purified polysaccharide, S-a3, whose antiviral mechanism is associated with the disruption of virus attachment to host cells. Additionally, S-a3 has significantly inhibited cell death and viral gene expression in viral infected Vero cells, and alleviated viral induced body weight loss, death, and paralysis in a transgenic mouse model.[19]

The treatment technique (herbal dosing by rectal enema) designed for this study was unique and allowed application of an herb to a vomiting patient. The test herbal medicine formula, ZLT, was mixed with warm water using a consistent dose based on body weight (0.2 g/kg, twice daily) and was administered with a soft rubber catheter. This was followed by 10 minutes of gently pressing against the anus with a cotton ball or towels. This technique was very well tolerated by the canine patients. To the investigators' surprise, this approach to herbal dispensary did not cause any increased tenesmus or bowel movements in dogs affected with diarrhea from CPV enteritis. The fact that the herbal ZLT treatment group had an improved recovery rate compared to the Control Group demonstrates that dosing herbal medicine formulations by rectal enema is a viable method to give herbal formulas to patients that can't be dosed by the oral route. The herbal medicine, ZLT, when purchased in bulk powder was cost effective, even for animal shelters at about US$0.30 per gram.

The present study had several limitations. The study enrolled a total of 19 subjects which was less than the goal of 24 subjects (12 per group), which was initially calculated to give a 90% power to reject the H_0 (0.05 significance level). The enrolled sample size of 19 subjects reduced the power to approximately 74% which restricted statistical evaluation. The cause of this was multifactorial: (1) this was a single site study, (2) an infectious respiratory disease outbreak occurred at the shelter during conduct of the study, and (3) a large number of subjects were rejected due to inclusion restrictions.

Additionally, the study was not blinded. Potential for bias from personnel recording clinical signs, therefore, cannot be ruled out. The study attempted to reduce bias to a minimum by doing the following: (1) each subject was housed separately in the isolation area so indices such as presence of feces or vomitus was clear for each subject; (2) each kennel was thoroughly cleaned twice daily for optimal detection of excrement or vomitus; (3) the recording of subject clinical signs was performed by different people each day before the herbal medicine treatments. This made it less clear to personnel whether a patient was in the Control or Integrative Treatment Group at the time of recording. Data collection considered not significantly affected by bias included the presence/absence of vomitus and/or diarrhea, whether a subject was voluntarily eating and patient mortality. The indices that were more likely to be affected by lack of blinding included the subject's energy level (i.e. lethargy) and stool quality (i.e. hematochezia). Post hoc examinations of this data revealed no evidence of significant difference between the two groups: (1) lethargy: control 2.0±2.3 days vs. integrative 2.6±1.5 days; $p = 0.335$; (2) hematochezia: control 4.0±1.5 days vs. integrative 3.5±2.3 days; $p = 0.929$.

An unexpected study outcome was the similar recovery times (4-4.5 days) for both study groups. The dogs receiving ZLT had decreased mortality, so it was expected they would have a shorter mean recovery time when compared to controls. Larger study groups will be necessary to validate whether this is a true finding. The similar recovery times may be related to the intense nature of conventional treatment that both groups received. The recovery time of patients in the present study is comparable to the literature, which reports recovery time for inpatient treatments of CPV enteritis patients to be 4.6 days.[6]

In summary, based on the 19 patients enrolled in this pilot study, it was found that for naturally occurring CPV enteritis, a combination of ZLT and conventional treatment resulted in significantly lower mortality when compared to conventional treatment only patients. The Chinese herbal medicine, ZLT, was effectively administered to study dogs by rectal enema without adverse effects from the herbal medicine or administration technique. The cost of ZLT was estimated at US$0.30 per gram, when bulk purchased, making it a cost-effective treatment for rescue organizations and shelters. It is important to follow this study with blinded, prospective studies containing larger sample sizes to validate the findings. These studies could provide the scientific community with validation for the use of ZLT in a variety of clinical situations such as inpatient or outpatient treatment at veterinary hospitals, and application at shelters or other situations where conventional treatments and finances are limited. Based on the findings and under the experimental conditions of this study, ZLT is recommended as an adjunct to conventional treatment to lower mortality for dogs affected with CPV enteritis.

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FOOTNOTES

[a] Panacur, Intervet/Merck Animal Health, Rahway, NJ, USA
[b] R version 3.2.2, The R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org
[c] Unasyn, Roaring Division of Pfizer Inc, New York, NY, USA
[d] Ampicillin, Boehringer Ingelheim Animal Health USA Inc, Duluth, GA, USA
[e] Baytril, Bayer HealthCare LLC, Animal Health Division, Shawnee Mission, KS, USA
[f] Cerenia, Zoetis Inc, Kalamazoo, MI, USA
[g] Propectalin Paste, Vetoquinol, Fort Worth, TX, USA
[h] Multipurpose Red Rubber catheters, Sizes 8-10 French, Jorgensen Laboratories Inc, Loveland, CO, USA

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