

Clinical Studies

Efficacy of GV-1 Aqua-acupuncture for Acute Diarrhea in Dogs: A Controlled, Randomized Clinical Study

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ABSTRACT

The objective of this randomized controlled study was to determine whether integrating GV-1 aqua-acupuncture (aqua-AP) and conventional therapy can accelerate the resolution of acute diarrhea in dogs. A total of 39 client-owned dogs experiencing acute diarrhea of less than 5 days duration were enrolled and randomly assigned to Group A, conventionally treated control (no aqua-AP); Group B, conventionally treated control plus sham aqua-AP (subcutaneous injection of vitamin B12 at a non-acupuncture point); or Group C, conventionally treated plus aqua-AP at GV-1 (vitamin B12 injected at GV-1 acupuncture point). Conventional treatment, uniformly applied to all patients, consisted of a bland diet, metronidazole and probiotics. Each owner, blinded to the group assignment of their dog, reported the time duration from the start of the treatment to the first observed normal stool (denoted as T_{ns}). Study results revealed a significant difference among the T_{ns} values of the 3 study groups. The mean $\pm$ SD T_{ns} in Group A was 68.0 $\pm$ 59.5 hours and 59.5 $\pm$ 41.6 hours in Group B. Group C was 22.6 $\pm$ 28.6 hours, which was more than 50% shorter than each of the other 2 groups. Furthermore, the proportion of subjects in Group C, whose first stool after treatment initiation was normal, was significantly greater than the other groups (A: 7.7%; B: 7.7%, and C 46.2%; $p = 0.029$). These findings suggest that aqua-AP at GV-1 can be an effective integrated treatment with conventional therapy for resolving acute diarrhea in dogs. Further studies with a larger sample size, and comparisons with other conventional treatments are warranted.

Keywords: acute diarrhea, aqua-acupuncture, canine, GV-1, integrative medicine

ABBREVIATIONS: Aqua-AP: aqua-acupuncture; GV-1: Governing Vessel-1; NS: number of stools; T_{ns} : first observed normal stool; TCM: traditional Chinese medicine

Acute diarrhea is a common occurrence among canine patients in small animal general practice.^{1,2} A Banfield Pet Hospital *State of Health Report* in 2016 ranked gastroenteritis and colitis each within the top 20 diagnoses for dogs, occurring most commonly in dogs 3 years of age and under.¹ Acute diarrhea is defined as lasting 14 days or less, while chronic diarrhea is defined as lasting longer than 2 weeks.³ Canine diarrhea can be further characterized as small bowel (e.g. normal-mild frequency increase, increased volume, absent mucus and tenesmus) versus large bowel (e.g. markedly increased frequency with tenesmus/mucus, decreased volume); but it is not uncommon for both to be present.^{1,2}

Canine acute diarrhea is characterized by increased fecal fluidity with most cases mild and self-limiting, requiring minimal diagnostics.^{2,3} The most common causes of self-limiting diarrhea include dietary indiscretion, drugs, stress, excessive exercise, parasites and viruses.³ Examples of dietary indiscretion include rapid diet change, foreign material, table scraps/garbage, plants,

chemicals or toxins.² More life-threatening causes of acute diarrhea include canine parvovirus, severe pancreatitis, acute liver disease, hypoadrenocorticism and intussusception.³ These more serious causes are usually accompanied by severe vomiting, dehydration, lethargy and abdominal pain, with standard outpatient treatment (metronidazole, probiotics) less likely.³

Most cases of self-limiting diarrhea resolve within 1-5 days of removing the underlying etiology and/or symptomatic treatment.³ The standard treatment for this condition includes a bland diet, metronidazole and probiotics. In a review of dogs admitted to the University

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of Liverpool Small Animal Teaching Hospital between January 2000 and December 2008 with diarrhea as one of their clinical signs, clinicians prescribed antibiotics in 71% of cases, followed by 26% of cases prescribed probiotics, prebiotics, absorbent and anti-motility drugs.⁴

Acupuncture is one of the oldest modalities of traditional Chinese medicine (TCM), dating back more than 3,000 years.⁵ It is currently endorsed by the National Institutes of Health for many internal medicine conditions including those involving the gastrointestinal tract. Scientific research into the mechanisms and benefits of acupuncture for gastrointestinal disorders has been rapidly growing in the last 30 years.⁵ Studies have demonstrated that acupuncture could decrease disease severity and inflammation via increased vagal activity in inflammatory bowel disease, and could play beneficial roles in the regulation of gut dysbiosis, visceral pain, motility and intestinal barrier function.⁵

Governing Vessel-1 (GV-1, *Chang-qiang*), a commonly used acupuncture point, is located on the midline in a depression dorsal to the anus and ventral the tail.⁶ Needle insertion is perpendicular and depth is 0.5-1 “*cun*” (*cun* = width of patient’s last rib).⁶ In a study conducted at Seoul National University, a novel dye (Alcian Blue) was able to trace the Governing Vessel Meridian of a rat injected at *Bai-hui* (lumbosacral space).⁷ The study showed that, after injection, the dye flowed along the Meridian from the acupuncture point, while it spread by diffusion process when injected at non-acupoints.⁷

Aqua-acupuncture (aqua-AP), or the injection of a substrate into the GV-1 acupuncture point, has had limited studies. One recent study looked at aqua-AP at GV-1 with regards to post-operative pain in horses.⁸ This 2020 study investigated GV-1 as an administration point for flunixin meglumine analgesic injection following castration, compared to intravenous dosing in 20 horses.⁸ The study compared the effects of injecting 1/10th drug dose at the acupoint to the effects of injection using conventional routes at larger doses.⁸ By examining pain scores and other physiology parameters, the study found injecting flunixin meglumine at 0.11mg/kg at GV-1 was as effective as injecting the higher dose of 1.1mg/kg of flunixin meglumine intravenously.⁸

Currently, there has been limited study of aqua-AP at GV-1 to improve diarrhea in dogs. Although one study showed that dry needle stimulation at the GV-1 acupuncture point affected colonic motility in dogs, its use in small animal clinical practice has yet to be reported.⁹ The objective of this study was to determine whether incorporating aqua-AP

at GV-1 along with conventional treatment would provide more efficient resolution of acute diarrhea in dogs than conventional treatment only. The study hypothesized that patients receiving the proposed aqua-AP treatment at GV-1, along with conventional therapy, would have clinical signs of diarrhea resolve sooner than those receiving conventional treatment only, or receiving a vitamin B12 injection at a non-acupuncture (sham) point.

MATERIALS AND METHODS

Animals

The study population consisted of dogs experiencing acute diarrhea recruited from Bridgewater Veterinary Hospital in Bridgewater, New Jersey, USA and from the surrounding areas. The inclusion criteria included: 1) aged 12 months or older; 2) experiencing diarrhea with a stool consistency of Type 5, 6 or 7 (Bristol Stool Chart), lasting no more than 5 days; and 3) with minimal vomiting and no need for hospitalization, subcutaneous fluids or antiemetics (Figure 1).¹⁰ Patients infected with parvovirus were excluded due to the severity of the disease and different standard of treatment for this disease from the study protocol. Verbal informed consent was obtained from the owners of participating dogs.

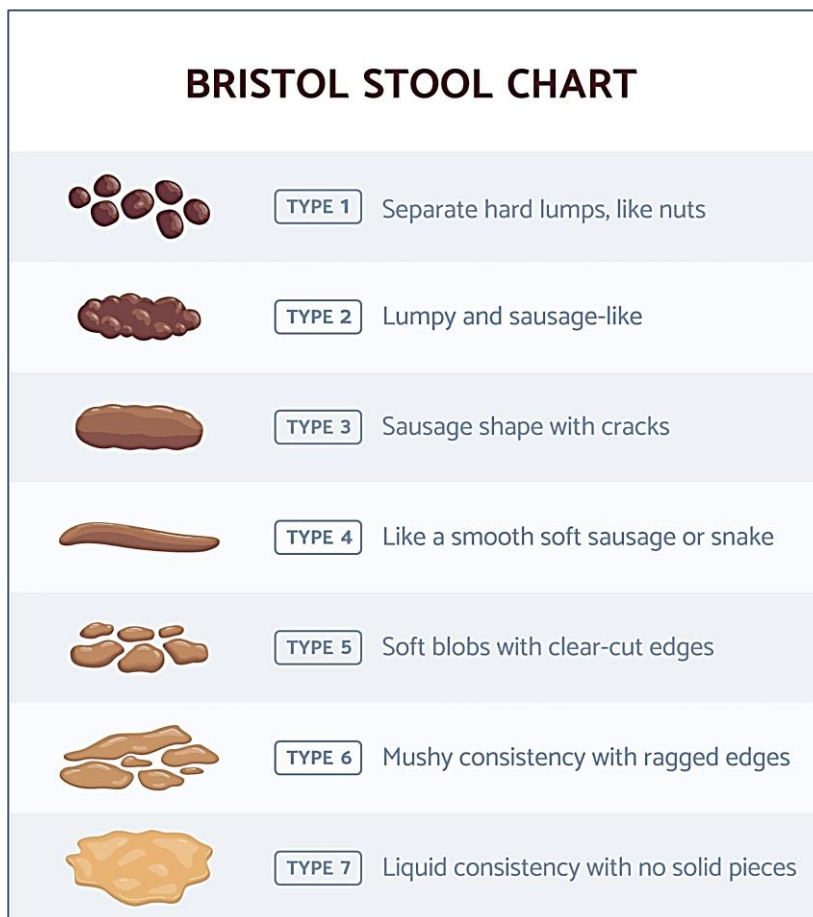


Figure 1: The Bristol Stool Chart is a pictorial diagram used to classify a study subject's stool quality.

Treatment Groups and Procedure

Enrolled subjects were randomly assigned to 1 of 3 groups by drawing an assigned treatment group out of a hat: Group A, conventionally treated control (no aqua-AP); Group B, conventionally treated control plus sham aqua-AP (subcutaneous injection of vitamin B12^a over the right shoulder in front of the scapula); or Group C, conventional treatment plus aqua-AP (vitamin B12^a) injected at GV-1 acupuncture point (midline in a depression dorsal to the anus and ventral to the tail).⁶ Each dog was taken away from their owner for treatment to ensure that the owner stayed blinded to the treatment group. The injections were administered by a certified veterinary acupuncturist with the volume injected based on body weight (Table 1).

Conventional treatment for all enrolled patients included metronidazole^b, dosed at 12 mg/kg [rounded up or down to closest half tablet size (50mg/250mg/500mg)] twice daily, with food for 10 days. Patients were also given a once daily probiotic capsule^c (given at least 2 hours after evening meal). All enrolled patients were given a bland diet for 5 days. A list of foods was provided to the owners as approved options for a bland diet. The list included both canned/dry standard commercial diets^{d,e} or a homemade bland diet containing boiled chicken breast with skin and fat removed, white rice, sweet potatoes, unflavored cooked oatmeal, boiled 90% lean ground beef pattied dry, and scrambled eggs with no butter, cheese or milk. Although starting with small frequent meals was encouraged, owners were given autonomy to determine the ratio of ingredients, quantity, and frequency of whichever diet they chose. After 5 days of bland food, the subjects were gradually weaned back to their regular diet over the course of another 2 to 4 days.

Outcome Measurements

Each client, blinded to their dog's treatment group, was called for a progress update the day following the appointment and again 10 days later. After 10 days, each client returned a worksheet recording the time and date of their pet's first normal stool (Type 4 or lower, Bristol Stool Chart), and how many stools their pet had per day over 10 days. Improvement was measured based upon the time frame to normal feces in hours starting from the time when the study treatment was received.

The primary outcome measurement for testing the study hypothesis was the time duration (in hours) from the appointment until the patient had a normal stool (ns) rating (T_{ns}) of Type 4 or better. Owners recorded daily stool data, as well as T_{ns}, on a standardized worksheet (Figure 2). If a patient's first stool

after treatment was normal, the outcome for that patient was designated as zero. A zero time duration noted immediate clinical resolution following the assigned treatment with no further symptoms of diarrhea. Stools per day were also counted, as decreased frequency can be a good indicator of resolution of colitis. The day of treatment was considered Day 1 and was documented through Day 10 to test the hypothesis (i.e. lower day number for normal stool = faster resolution).

Table 1: Volume of vitamin B12^a, based on body weight, injected into the GV-1 or the sham acupuncture point.

Patient Body Weight	Volume Vitamin B12 Injected (1000 mcg/mL)
0-9.9#	0.5 cc
10-24.9#	1 cc
25-49.9#	1.5 cc
≥50#	2 cc

CLIENT DATA COLLECTION FORM

Date of Appointment: _____

Time of Appointment: _____

Bristol Stool Chart

Date of First Normal Stool: _____

Time of First Normal Stool: _____

DAY	Type and Number of Stools
Day 1 (Appointment)	
Day 2	
Day 3	
Day 4	
Day 5	
Day 6	
Day 7	
Day 8	
Day 9	
Day 10	

Figure 2: Client Data Collection Form

Statistical Analysis

The hypothesis was that patients receiving aqua-AP with vitamin B12 at GV-1 would have shorter T_{ns} than patients receiving no aqua-AP or sham aqua-AP with vitamin B12. Based on the outcome measurements for comparison of the 3 groups, the study tested the H_0 (mean T_{ns} not different among 3 treatment groups) vs. H_A (mean T_{ns} different among 3 treatment groups). As T_{ns} data were not normally distributed, nonparametric one-way ANOVA (i.e. Kruskal-Wallis test), was used. When the H_0 was rejected, the inference proceeded with the post hoc analysis to determine whether the test group (Group C) had shorter T_{ns} than the other 2 control groups, respectively, using Wilcoxon rank sum test with Bonferroni corrections. A significance level of 0.05 was used for the Kruskal-Wallis test, whereas an adjusted significance level of 0.017 (0.05/3) was used for the post hoc pairwise Wilcoxon rank sum test.

To obtain a 90% power for the Kruskal-Wallis test in order to reject the null hypothesis with a significance level of 0.05 when the effect size $\delta/\sigma = 1.5$ (δ = minimal group meaningful difference; σ = overall standard deviation), a sample size of at least 14 subjects per treatment group (i.e. 42 total) was required. All data graphic presentations and statistical analysis were performed using a commercial statistical software^f.

RESULTS

A total of 39 dogs experiencing acute diarrhea within 5 days of their clinic visit satisfied study inclusion/exclusion criteria and were enrolled in the study. All subjects completed the 10-day clinical trial. There were no statistically significant differences in mean age, body weight or sex (all $p > 0.05$) between study groups (Table 2). Non-statistical differences noted were: 1) Group A subjects were younger than those in the other 2 groups; and 2) Group C had a lower proportion of female subjects than the other 2 groups. Each group contained a variety of breeds (A = 9 + 2 mixed-breed; B = 12; C = 10). There were no adverse effects from any study procedure, both conventional treatments and aqua-AP. The randomization procedure resulted in 13 subjects being assigned to each of the treatment groups. The slightly smaller sample size than planned reduced the power to 88%.

Owner-reported T_{ns} for aqua-AP at GV-1 (Group C) was compared to the 2 control treatments (no aqua-AP/Group A and sham aqua-AP/Group B) to determine efficacy for improving canine acute diarrhea. The mean $\pm$ SD T_{ns} in Group A was 68.0 $\pm$ 59.5 hours, 59.5 $\pm$ 41.6 hours in Group B, and was 22.6 $\pm$ 28.6 hours in Group C, which was more than 50% shorter than each of the other 2 groups (Figure 3). Based on Kruskal-Wallis test, there existed a statistically significant difference among the 3 treatment groups ($p = 0.039$), which suggests that the treatment factor had an overall effect on the group T_{ns} outcomes. Post hoc pairwise group comparison reveals that Group C's T_{ns} was significantly smaller than that of Group A ($p = 0.013$) and of Group B ($p = 0.015$). The T_{ns} values were not significantly different between Group A and Group B ($p = 0.972$).

Counting the subjects whose first stool after the treatment was normal, the observations were: 1 dog in Group A (7.7%); 1 dog in Group B (7.7%), and 6 dogs in Group C (46.2%). The difference of proportion between Group C and Group A/B, was statistically significant ($p = 0.029$).

The owners also reported number of stools per day (NS) for 10 days after receiving the treatment. On 6 out of 10 days, Group A had the greatest mean NS among the treatment groups. The mean $\pm$ SD (over subjects) maximal NS (over 10 days) in Group A was 4.15 $\pm$ 1.77, in Group B was 3.46 $\pm$ 1.71, and in Group C was 3.31 $\pm$ 2.10. The NS values did not have a statistically significant difference when compared among the 3 study groups on any of the 10 days (range, $p = 0.190 \sim 0.859$).

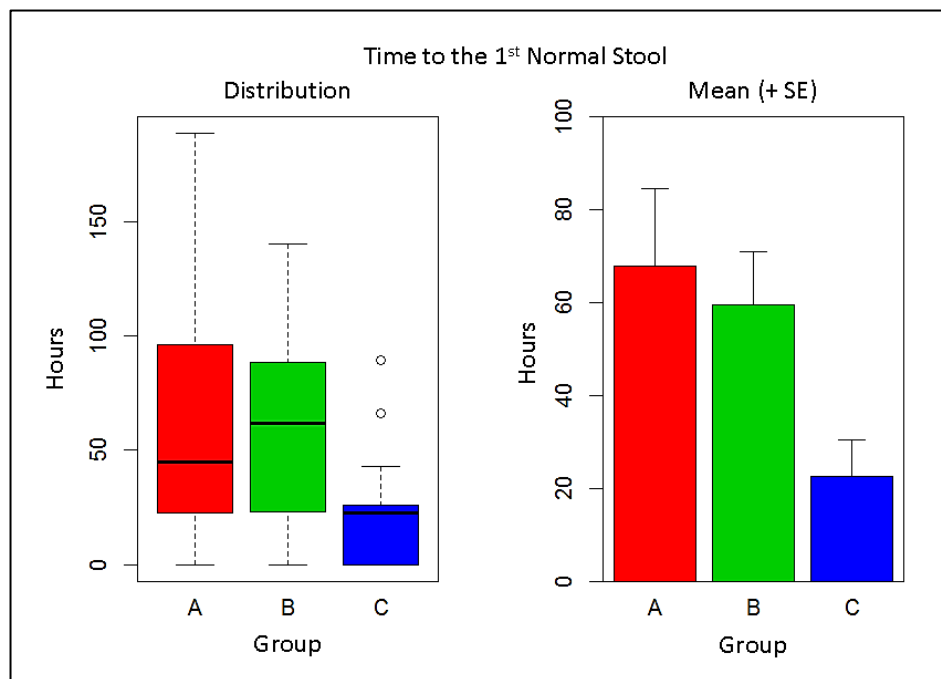


Figure 3: Distribution (left) and group mean (right, + standard error bar) of the 3 study groups, demonstrating Group C's significantly ($p = 0.039$) shorter treatment time to 1st observed normal stool

Table 2: Summary statistics of subject's signalment data; age and body weight are presented with mean±SD in the table; *p*-values for age and weight are overall group difference by Kruskal-Wallis test; % female is overall group difference by Fisher's Exact test.

	Group A	Group B	Group C	<i>p</i> -value
Age (yrs)	3.2 ± 3.1	6.5 ± 3.8	5.4 ± 4.0	0.071
Weight (lb)	44.6 ± 26.1	37.1 ± 29.3	36.1 ± 27.2	0.578
% Female	53.8% (7/13)	61.5% (8/13)	23.1% (3/13)	0.135

DISCUSSION

The findings from this randomized, controlled and owner blinded clinical study suggests that aqua-acupuncture at GV-1 can be an effective treatment for improving clinical signs of acute diarrhea. Patients that received vitamin B12 aqua-AP at GV-1 had a statistically significant faster resolution of their diarrhea and were more likely to have a normal stool following initial treatment compared to patients that received vitamin B12 injection at a sham acupuncture point or conventional treatment only.

In traditional Chinese medicine, the Governing Vessel or *Du* Channel originates in the lower abdomen, emerges from the perineum and passes through the coccygeal vertebrae.¹¹ From there, it runs upward along the middle of the spinal column and reaches the head to penetrate the brain.^{6,11} It continues up to the vertex of the head and descends across the forehead and nose and terminates inside the upper gum at GV-28.¹¹ As it courses along the dorsal midline, the GV connects all six *Yang* Channels (Large Intestine, Stomach, Small Intestine, Bladder, Triple Heater and Gallbladder), spinal cord and brain. The Governing Vessel is “the sea of all *Yang* Channels,” and its connection allows the GV to govern the *Qi* of all the *Yang* Channels.¹¹ The GV Meridian, therefore, has traditionally been used for diarrhea, constipation, and perianal problems, as well as epilepsy.¹¹

The aqua-AP technique emerged in the 1950s in China and has been widely used in TCM as a method of acupuncture point stimulation. It is considered to have a more sustained effect than dry needle stimulation, combining the effect of acupoint stimulation and the effect of the substance injected.^{12,13} It appears to create an effect by local spatial configuration changes within an acupuncture point location, which, along with the liquid substrate, appears to stimulate neuronal activity.¹² The technique may be performed with a variety of substances that include normal saline, vitamin B12 (as in the present study), the patient's blood, medicinal herbs and various drugs. There are several positive attributes that support the use of aqua-AP in dogs with diarrhea visiting the veterinary clinic. It is a simple and inexpensive procedure without likely side-effects, does not require the patient to be restrained once aqua-AP is performed (unlike dry needle AP) and vitamin B12 is considered a *Qi* tonic.¹⁴ This makes it useful for concurrent TCVM Patterns such as Spleen *Qi* Deficiency, which is an important consideration in dogs presenting with diarrhea.¹⁵

Although aqua-AP at GV-1 has not previously been studied in canines, Kim et al. demonstrated that acupuncture at GV-1 in canines depressed the proximal colonic motility

by decreasing the total duration and the frequency of contractile states ($p < 0.05$).⁹ This decrease in hypermotility may contribute to the anti-diarrheal clinical effects of GV-1 stimulation.⁹

Previous studies in other species have also shown similar findings. Park et al. investigated the effect of acupuncture treatment of young pigs with induced enteropathogenic *Escherichia coli* diarrhea.¹⁶ This research compared 21-day-old piglets treated once daily for 3 days: 10 treated with intramuscular injections of enrofloxacin and 10 treated with dry needle (26g) acupuncture at GV-1.¹⁶ The remaining 12 piglets served as normal controls ($n=2$) and diarrhea controls ($n=10$).¹⁶ By day 6, all treated piglets, whether treated with acupuncture or antibiotics, had recovered.¹⁶ The histopathology of colon and stomach mucosa in all treated piglets was relatively similar to those of the normal controls, while untreated control piglets had severe necrotizing inflammation of the fundic stomach with fundic gland architecture destruction.¹⁶ This study demonstrated that acupuncture treatment can be as effective as antibiotic treatment for control of induced *E. coli* diarrhea in piglets at its early stages (i.e. the onset).¹⁶

In a clinical study with yet another species, Choi et al. investigated the use of aqua-AP at GV-1 for treatment of viral diarrhea in young calves ($n=11$). The authors of this small study concluded that the use of aqua-AP technique at GV-1 was an effective cure for severe watery viral associated diarrhea in young calves.¹⁷ This study was limited, however, by small animal numbers and lack of a control group.¹⁷

In the present study, there were several patients (4 out of 13) in the GV-1 treatment group who had improvement to normal stool or normal stool immediately following the vitamin B12 injection. This immediate clinical resolution, however, was followed by decreasing quality of stools (i.e. stool score increase) over a period of 1 to 3 days. A study that may shed light on this change was conducted by Ma et al. The mechanism of single point acupuncture of ST-36, BL-25 or GV-1 on acute diarrhea in a rabbit model was investigated.¹⁸ This study looked at duration of the needle stimulation effect, as well as the positive therapeutic effects of acupuncture on herb-induced diarrhea. A single needle at BL-25 or GV-1 decreased the fecal water rate on day 1 ($p < 0.01$), but was no longer significant when compared with the control group on the following days, similar to some subjects in the present study.¹⁸ Interestingly, single point acupuncture at bilateral ST-36 significantly decreased the fecal water rate on day 1 with continuous improvement between days 3 and 7, even reaching the same level as the control group.¹⁸ Additionally, ST-36 was the best of the 3 acupoints to

improve weight loss, diarrhea severity grade and alleviate depression, as well as affecting GI motility and hormones. These results may explain why some cases of acute canine diarrhea in this study improved initially but then regressed. In the future, aqua-AP at ST-36 in a canine model may improve on the present study's results and the effects may be sustained over a longer period.

Study findings demonstrated that a conventional approach consisting of bland diet, probiotics and metronidazole has limitations. Fowler et al. showed that in cases of stress colitis using metronidazole only, there was an 89% response rate (to normal stools) within 10 doses compared to *Da Xiang Lian Wan* which showed a 97% response rate ($p < 0.05$, non-inferiority test).¹⁹ The Fowler study, along with findings from the present study, bring into question whether metronidazole, probiotics and bland food is the best treatment. Future studies using *Da Xiang Lian Wan* and GV-1 aqua-AP should be considered.

The present study had several limitations. These included variability of stools at study start (ranging from watery to soft, mucus covered), possible recall errors for several owners contacted to provide missing data, investigators not blinded, and variable timing for the first dose of metronidazole after the initial appointment. The most important of these limitations was the lack of blinding of the investigators compiling study data. Nonetheless, the owners documenting the stool types and duration of diarrhea were blinded to the study groups, which helped to avoid interpretation bias.

Recruitment of enough dogs for the study to provide meaningful statistics was also a limiting factor. Most acute diarrhea occurs in very young dogs. One study noted that risk for diarrhea decreased from 16% in 7-to-12-week old puppies to 5.4% in 12-to-18-month-old dogs.³ There was concern that parasitism in very young study participants might confound study findings, therefore, inclusion criteria limited dogs to 12 months or older. Only 1 dog (control, Group A) was diagnosed with a parasite infection (2+ hookworms). This subject improved with conventional treatment alone, as the owner did not immediately pick up the dewormer, and the dog had a T_{ns} of 22.5 hours. Although there was only 1 case with parasitism, study findings suggest this may not significantly affect results. Study enrollment could have therefore been opened to all age groups and may be a consideration for future studies to improve numbers of enrolled dogs.

In summary, the present study found that dogs experiencing acute diarrhea of less than 5 days duration had significantly shorter duration of their diarrhea and time to first normal stool, when aqua-AP at GV-1 was combined with conventional medical treatment (metronidazole, probiotics, bland diet). Based on these study findings, the addition of aqua-AP at GV-1 can offer more effective resolution of acute diarrhea in dogs and is recommended as a beneficial adjunct to conventional treatment. Further prospective blinded investigations with larger sample size and comparisons with other conventional treatments are warranted to substantiate the findings of this study and document the clinical usefulness of this acupuncture technique for treatment of acute diarrhea in dogs.

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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 or funding from any organization in the public, commercial or non-profit sectors.

FOOTNOTES

- ^a Vitamin B-12 VetOne, MWI, Boise, Idaho, USA
- ^b Metronidazole, generic, Unichem, East Brunswick, NJ, USA
- ^c Provable-Forte®, Nutramax Laboratories, Lancaster, SC, USA
- ^d I/D, I/D Low Fat (canned or dry), Hill's Pet Nutrition, Topeka, KS, USA
- ^e W/D (canned or dry), Hill's Pet Nutrition, Topeka, KS, USA
- ^f R version 3.5.2. The R Foundation for Statistical Computing, Vienna Austria (<http://www.R-project.org>)

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