Preoperative Oral Administration of *Yunnan Baiyao* and Its Effect on Coagulation Parameters in Tick-Borne Disease and/or Heartworm Seropositive Dogs: A Pilot Study

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ABSTRACT
The overall goal of this clinically focused pilot study was to investigate the preoperative benefit of oral administration of *Yunnan Baiyao* (YB) to *Ehrlichia. canis*, *Anaplasma platys* and/or heartworm seropositive canines. A total of 12 clinically normal dogs undergoing sterilization procedures were randomly subdivided into Control/Placebo Group (n=6) or Treatment Group (n=6). Each patient received three oral doses of YB (0.5g/5kg) or placebo every twelve hours with the last dose given the evening before surgery. Citrated blood measurements for prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen were obtained before the first YB dose and after the third dose. Intraoperative blood loss was estimated by blood-soaked gauze sponge counts. The study found the Control Group's mean aPTT increased by 3.8% while the Treatment Group's decreased by 0.42%. PT values were slightly decreased in both groups. Fibrinogen decreased in controls (9.7%) but had a modest increase in treated dogs (12.4%). Intraoperative blood loss was increased in controls (3%) compared to the Treatment Group (mean±SD: 6.6±6.8 vs. 6.4±3.6, respectively). Findings from the study which suggest preoperative benefits from YB administration to seropositive dogs include stabilizing aPTT and fibrinogen values along with reduced intraoperative blood loss in YB treated dogs. Although these modest findings were not statistically significant in this small study, the biological trends suggest potential benefits of preoperative administration of YB before routine elective surgeries in seropositive patients and support a clinical trial with larger sample size.

Keywords: *Yunnan Baiyao*, ovariohysterectomy, castration, blood loss, coagulation parameters, tick-borne disease, heartworm disease

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ABBREVIATIONS

aPTT	Activated prothrombin time
EACA	Epsilon aminocaproic acid
PT	Prothrombin time
TA	Tranexamic acid
YB	*Yunnan Baiyao*

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Professional Certifications: CVA, CVCH (Rodriguez)

Veterinarians and veterinary students perform sterilization procedures, namely ovariohysterectomies (OVH) and orchiectomies, routinely all over the globe. These are standard surgeries, yet blood loss factors need to be taken into consideration such as patient preoperative status, concurrent diseases and surgeon technique.[1] The concern for excessive bleeding is increased for veterinary patients seropositive for tick-borne diseases and/or heartworm disease. The rickettsiae *E. canis* and *A. platys* are transmitted by ticks, and it has been demonstrated that dogs who are seropositive for both have a greater tendency to ooze and bleed during surgery in comparison with seronegative patients.[2] Identifying a treatment which can reduce the excessive bleeding tendency in these seropositive patients, particularly in areas of the world that have high infectivity rates with these organisms, would be beneficial. In Grenada, there is a high prevalence of tick-borne disease, and experiential

evidence has found that it is appropriate to do surgery on these seropositive patients once their platelet counts and hematocrit levels (platelets >150,000, HCT >30%) as well as other preoperative criteria for the surgical lab are within acceptable limits. This becomes of note since some patients who are seropositive may not come back if rejected for surgery, thus reducing the chance for population control. Patients who are evaluated for surgery and fall below the acceptable ranges are treated for disease before being considered for surgery. Subclinical seropositive patients who undergo surgery are normally sent home with treatment post-operatively.

Conventional hemostatic agents commonly used peri- and intraoperatively in veterinary medicine include the antifibrinolytic drugs tranexamic acid (TA) and epsilon aminocaproic acid (EACA).[3-8] The drug TA is available as an injectable solution and may also be used topically. Common side effects include vomiting due to hypotension and a risk of thrombosis, which present a shortcoming when considering administering the drug to a patient.[1] Epsilon aminocaproic acid can be administered orally or IV with side effects that may include hypotension and thrombi generation. It is therefore important to weigh the benefits over the complications.[4]

The herbal formula *Yunnan Baiyao* (YB) originated in the Yunnan Province, China and was developed by Doctor Qu Huan-Zhang around 1902 for the purpose of removing blood stasis, stopping bleeding, relieving pain, detoxifying, and reducing swelling.[9] Its hemostatic effects and safety have been demonstrated across multiple species. Both oral and topical application of YB significantly shortened bleeding times in rats and time to blood clot formation in rabbits and humans.[10] It has also been widely used during times of war to treat injuries.[9] It is commonly used in traditional Chinese medicine (TCM) and traditional Chinese veterinary medicine (TCVM) as it has hemostatic indications and is known to cause fewer side effects (rare mild diarrhea) than its conventional medicine counterparts, TA and EACA.[11,12]

Ladas et al. described the use of YB "in adolescents with cancer as an adjunct to uncontrolled bleeding in the palliative care setting" in a retrospective case-series report. YB solution significantly decreased clotting time when compared to saline control, starch and starch with calcium comparisons in rabbit and human blood.[13] Results in a canine study investigating blood loss and coagulation following nasal biopsy in YB treated dogs and controls demonstrated that the time to stop bleeding was significantly shorter ($p<0.05$) in the YB group when compared to controls and was not influenced by underlying disease. There was also decreased blood loss as a percent of body weight when YB treated dogs were compared to controls (14% vs 25% respectively).[10] No study has explored the effects of administration of YB to *E. canis*, *A. platys*, and/or heartworm seropositive dogs.

The objective of this pilot study was to explore the potential of preoperative oral administration of YB in reducing intraoperative bleeding in dogs who were seropositive to tick-borne diseases and/or heartworm disease, as well as blood coagulation parameters, PT, aPTT and fibrinogen. The significance of finding an effective treatment is supported by studies demonstrating increased perioperative bleeding/oozing tendency in these dogs, even if they do not show clinical signs. It was hypothesized that a small pilot study would be an effective use of resources to evaluate YB's potential as an effective hemostatic agent for parasitized dogs and if positive effects were observed (blood loss reduction and/or coagulation parameter changes), then a larger clinical trial could be recommended.

MATERIALS AND METHODS

The subject population under investigation in this study was intact canines presenting to the St. George's University School of Veterinary Medicine Junior Surgery and Anesthesia Laboratory (SGUSVM JSAL) for elective sterilization procedures. The recruited study patients were predominately Grenada's indigenous mongrel breed (Pothounds) from different parishes. The SGUSVM had an existing partnership with the organization Pothounds Against Pregnancy (PAP) at the time of data collection. The organization traveled around the island and recruited patients that were transported to the JSAL for evaluation and admission for elective sterilization surgery. With

Table 1: St. George's University School of Veterinary Medicine Junior Surgery and Anesthesia Laboratory Premedication Protocol for elective sterilization procedures.

Orchiectomy Premedication		
Drug	Dose	Route
Acepromazine (10 mg/mL)	0.05 mg/kg	IM
Morphine (10 mg/mL)	0.3 mg.kg	IM
Ovariohysterectomy Premedication		
Drug	Dose	Route
Acepromazine (10 mg/mL)	0.05 mg/kg	IM
Morphine (10 mg/mL)	0.5 mg.kg	IM

Institutional Animal Care and Use Committee (IACUC) approval of the study, owners signed a unique research consent form to allow their pet to participate in this study. The inclusion criteria included: (1) age 6 months to 8 years; (2) male or female; (3) hematocrit > 29.5%; (4) Platelets > 150,000 × 10^3/L; and (5) SNAP 4Dx[a] seropositive for *Ehrlichia canis*, and/or *Anaplasma platys*, and/or heartworm disease. Patients were excluded from the study if (1) body condition score was < 2/5; and/or (2) had any disease process diagnosed by physical exam and/or blood work findings that prevented the patient from being fit for surgery.

All patients arrived between the Sunday and Tuesday afternoons preceding scheduled surgeries for Thursday of that week. All surgery patients were examined upon arrival by a JSAL clinician and approved for surgery after the selection criteria were confirmed. A complete physical examination, complete blood count (CBC), pre-anesthetic panel, and a SNAP 4Dx© test were performed on every patient. Enrolled patients were randomly divided into the seropositive Control/Placebo Group and seropositive Treatment Group. Randomization was conducted by writing down numbers on a piece of paper and randomly drawing from a pile and consequently assigning patients to one of the two study groups. The person drawing the numbers was blinded to the patients' medical record.

For patients in the Treatment Group, YB[b] was administered orally at a dose of 0.5g per 5kg twice a day (BID), and each patient received a total of three doses. Each 0.25g capsule of YB was placed in a pill pocket[c] and the appropriate dose was administered orally. The patients in the Control/Placebo Group received the appropriate number of empty pill pockets orally according to their weight (1 pill pocket per 5kg) without YB. The individuals administering the placebo or YB treatment were blinded to the patient's study group assignment.

Once the patients were enrolled in the study, the blood draw to obtain baseline coagulation parameters, PT, aPTT, and fibrinogen, was performed on Tuesday afternoon after the arrival of the last enrolled patient. A 3-cc syringe with a 22-gauge needle[d] was used to collect a 2-cc blood sample from the jugular vein. The blood was transferred to a 1.8 ml blue top tube containing sodium citrate by removing the needle from the syringe, removing the tube top, and dispensing the blood directly into the tube. The samples were immediately placed on a rocker. The rest of the samples were obtained in the same manner. Sample collection took approximately 15-30 minutes depending the patient's demeanor, cooperation, and number in the group.

The blood samples were taken to the SGUSVM clinical pathology laboratory and processed to obtain baseline PT, aPTT and fibrinogen parameters for each patient. The VetScan® VSPro analyzer[e] was used with the appropriate cartridges for each test. A total of 2-3 samples were processed at a time within a 1hour time block. The number of samples was contingent upon the number of patients recruited for the study that specific week.

Table 2: Study population age, sex, and seropositive status of study dogs.

Group / Dog Number	Sex	Age (years)	Weight (kg)	Seropositive Status
C / 1	M	5	16.4	Heartworm
C / 2	F	1	10.2	Ehrlichia
C / 3	F	3	19.2	Heartworm
C / 4	F	4	15.4	Ehrlichia
C / 5	F	3	14.7	Heartworm
C / 6	F	3	8.2	Ehrlichia+Heartworm
Overall	85.3% Female	mean±SD = 3.17±1.33	mean±SD = 14.0±4.08	
T / 1	F	4	14.6	Ehrlichia
T / 2	F	1.25	13.8	Heartworm
T / 3	F	5	10.0	Heartworm
T / 4	F	3	11.6	Heartworm
T / 5	F	5	10.3	Ehrlichia+Anaplasma
T / 6	F	1	10.4	Ehrlichia
Overall	100% Female	mean±SD = 3.21±1.78	mean±SD = 11.8±1.97	

C = Control group; T = Treatment group

After the patients received either three doses of the placebo or treatment YB (Tuesday night, Wednesday morning, Wednesday night), the post-administration blood sample collection and processing took place on Thursday morning at 8:15am before the scheduled surgery. Due to logistics involving the efficient running of the JSAL, the blood samples had to be collected after premedication of the patients (Table 1). After premedication of the patients and IV catheter placement, 2-cc of blood was collected directly from the IV catheter and into a 1.8 ml blue top tube containing sodium citrate, and the tube was immediately placed on a rocker. Sample collection time ranged from 10-15 minutes. The blood samples were then taken to the SGUSVM clinical pathology laboratory and processed to obtain post-dosing PT, aPTT, and fibrinogen parameters for each patient. The same procedure and equipment used for obtaining the baseline data was utilized.

In addition to the three coagulation parameters, the amount of blood loss during the surgery was estimated for each patient. Once the neuter or spay was performed, the assistant surgeon counted the gauze sponges used during the procedure. Calculation of estimated blood loss was done by counting how many 4" × 4" gauze sponges of the same material and brand were used intraoperatively and by visually estimating the degree of blood saturation of each gauze sponge, using the assumption that each fully saturated gauze sponge holds approximately 5 ml of blood.

Outcomes of the measurements, including those derived from pre-treatment data, post-treatment data, and their differences, were reported with summary statistics (mean±SD, median, % change). The Wilcoxon signed rank test was employed for testing the within-group changes (i.e. pre- vs. post-treatment) and the Wilcoxon rank sum test was applied for between-group comparisons. Significance level was set to be 0.05, although it is understood that, as a pilot study, the group sample size was small (n = 6) for any meaningful statistical significance test (power ≈ 65% and 40% for within-group and between-group tests, respectively). A commercial statistical software was used for all data graphic presentations and statistical analyses[f].

RESULTS

Based on the established criteria, a total of 12 dogs (1 male, 11 female) were enrolled in the study. All dogs were seropositive for either E. canis, *A. Platys* and/or heartworm disease: 6 heartworm positive, 4 Ehrlichia positive, 1 Ehrlichia/heartworm positive and 1 Ehrlichia/Anaplasma seropositive. Randomization resulted in 6 in the Control Group and 6 in the Treatment Group (Table 2).

The mean±SD age in the Control Group was 3.17±1.33 years versus 3.21±1.78 in the Treatment Group. The difference was not statistically significant between the two groups (p = 0.89). The mean±SD body weight in each study group was: 14.0±4.08 (control) and 11.8±1.97 (treatment), which was not significantly different (p = 0.31).

The mean±SD PT before treatment was 17.4±0.9 in the Control Group and was 17.2±0.6 in the Treatment Group (p = 0.85) (Tables 3 and 4). Both groups' PT was decreased after the treatment. The mean±SD values of PT change were -0.90±0.44 and -0.13±0.79 in the control and the treatment groups, respectively (Figure 1, Table 5). Based on Wilcoxon Signed Rank test, the change in the Control Group was statistically significant (p = 0.03) but was not significant in the Treatment group (p = 0.75). The between-group difference for PT changes was not statistically significant (p = 0.13).

The mean±SD aPPT before the treatment was 90.9±3.6 in the Control Group and was 89.9±10.1 in the Treatment Group (p = 0.59) (Tables 3 and 4). After treatment, the aPPT was increased in the Control Group, but was decreased in the Treatment group (Figure 2). The mean±SD values of aPTT change were 3.5±5.7 (p = 0.22) and -0.38±9.0 (p = 1.00) in the Control and the Treatment groups, respectively (Table 5). The between-group difference for aPTT changes was not statistically significant (p = 0.48).

The pre-treatment fibrinogen mean±SD was 3.62±1.91in the Control Group and was 2.58±0.82 in the Treatment Group (p = 0.97) (Tables 3 and 4). After treatment, fibrinogen was decreased in the Control Group

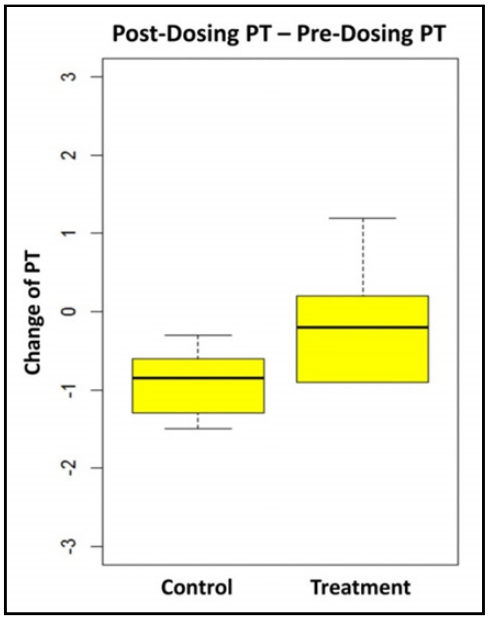

Figure 1: Distribution of prothrombin time change depicted by a box-plot for each of the study groups. The lower and upper bounds of the rectangle box are the 1st and the 3rd quartiles (Q1 and Q3), respectively, of the observed data. The thick horizontal line inside the box is the median. The whiskers (lines outside the box) are the highest and lowest observations. A box-plot without a bottom whisker (as in the present case) indicates that the 1st quartile (bottom of the rectangle box) is the same as the minimum in the Treatment group. This figure description also applies to Figures 2, 3 and 4.

(mean±SD change = -0.35±0.67, p = 0.34), but was increased in the Treatment group (mean±SD change = 0.32±0.18; p = 0.063) (Figure 3, Table 5). The between-group difference for fibrinogen changes was not statistically significant (p = 0.32).

Percent peri-operative blood loss was calculated by taking 8% of the patient body weight (total blood mL) and dividing the estimated blood loss by the total blood volume (Figure 4). The mean±SD values of blood loss were 6.60±6.78 in the Control Group and was 6.43±3.63 in Treatment Group (Tables 3-5). The between-group difference for blood loss was not statistically significant (p = 0.70).

DISCUSSION

The hemostatic effects and safety of *Yunnan Baiyao* have been demonstrated across multiple species along with its ability to control blood loss when given prior to surgical procedures.[10,12] The objective of this pilot study was to investigate the intraoperative hemostatic effects of presurgical administration of YB to dogs testing seropositive for tick-borne diseases (*E. canis*, *A. platys)* and/or heartworm disease. Intraoperative blood loss and changes in coagulation factors (PT, aPTT, fibrinogen) prior to surgery were evaluated in 6 patients receiving 3 doses of YB (last dose night before surgery) and 6 controls. Changes of interest included improved aPTT (quicker clot formation) in the YB treated dogs (reduction of 0.42%) while untreated seropositive dogs had an increased aPTT by 3.8%. Fibrinogen levels also displayed a positive change with increased fibrinogen value (12.4%) associated with YB treatment versus seropositive controls whose value decreased by 9.7%. The YB treated group also had less intraoperative blood loss (3%) than the untreated seropositive controls (mean±SD: 6.4±3.6 versus 6.6±6.8, respectively). The PT value decreased slightly in both groups. All beneficial changes noted with pre-surgery YB treatment in seropositive dogs suggested biological trends yet were not large enough to show statistical significance in the small group numbers utilized in this study. The findings do satisfy the hypothesis that this pilot study would demonstrate YB pre-surgical administration benefits and support a larger clinical trial to see if these findings can be replicated with statistically significant results.

The comparison of blood loss in seropositive dogs treated with YB to untreated seropositive dogs defines the most important objective of this study. This part of the investigation demonstrated that there is benefit in giving YB prior to surgery as there was a reduction in blood loss in these dogs. When investigating mechanisms of action with this Chinese herbal medicine, in addition to reduction of blood loss, there has been interest in the anti-inflammatory effects of YB. Of particular interest is its effects on the arachidonic acid metabolite pathways in acute inflammation rat model studies.[14] It has shown comparable activity in the carrageenan-induced rat paw edema model with the Cox-2 inhibitor, celecoxib. In addition, it demonstrated similar anti-inflammatory activity to the antihistamine, mizolastine, in the arachidonic acid induced inflammation model in which celecoxib is not effective.[14] In this same model, YB exerted statistically significant suppression of the leukotriene B4 (LTB4). This would be particularly beneficial to dogs with an injured vasculature from this inflammatory cytokine as it is a potent chemotactic factor for induction of leukocyte adhesion and injury to vascular endothelium. Ongoing chronic vascular injury from parasitism has been hypothesized to likely exacerbate bleeding during surgery in these dogs.[2]

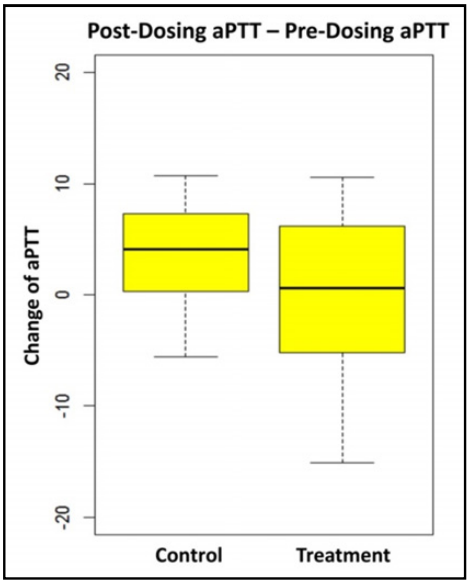

Figure 2: Distribution of activated prothrombin time change in each of the study groups.

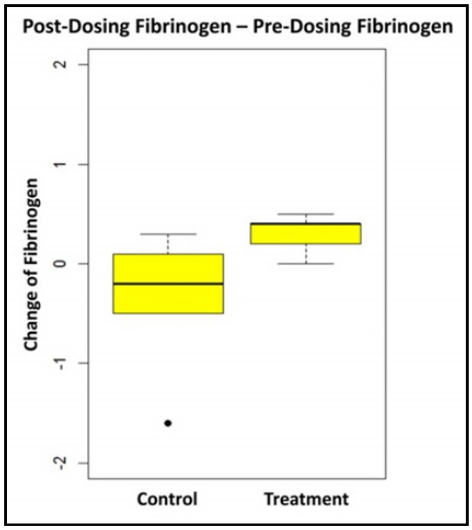

Figure 3: Distribution of fibrinogen change in each of the study groups. Note that, in the Control group, the small circle outside the box is an outlier, identified when the value is smaller than (Q1-1.5×IQR), where IQR = Q3-Q1.

Coagulation factors, namely aPTT, PT, and fibrinogen, were also of interest in the study patients undergoing surgery. Prothrombin time measures the extrinsic and common clotting pathways, and aPTT is an indicator of the intrinsic and common clotting pathways.[15] A significant trend was observed on aPTT changes in the study patients. This finding is relevant as Lanza et al. found that clinically normal appearing dogs who, however, are seropositive for *E. canis* and *A. platys* tend to bleed/ooze more during sterilization procedures. Their study did not find significant changes in PT and aPTT. The authors suggest these results may indicate that the normal battery of coagulation parameters are not effective in detecting a low-grade vasculitis contributing to the bleeding tendencies in these patients.[2] Moreover, the significant fact that aPTT changes (intrinsic coagulation pathway) are influenced by a patient's seropositive status to *E. canis* may help explain why these dogs bleed/ooze more during routine elective spays and neuters. The trend demonstrated in the present study, although not significant, points to the possibility that YB treatment may be beneficial in keeping aPTT values nearly unchanged.

Fibrinogen is an acute phase protein which plays a part in the inflammatory and coagulation processes.[16] Although fibrinogen levels have not been widely measured in dogs pre-operatively due to the consideration that they are more predictive of inflammation than coagulation competence, several human studies have shed a new light on this. A study by Walden et. al. compared fibrinogen levels, which ranged between low to high normal reference range, in 2000 patients prior to cardiac surgery and found an inverse relationship to bleeding during surgery (i.e. the lower the pre-operative fibrinogen, the more blood loss was recorded). The authors note that although fibrinogen is mostly considered as an acute phase/inflammation marker; they suggest that fibrinogen concentration prior to surgery may be more important than previously thought.[17] In the present study, fibrinogen remained nearly unchanged in the untreated controls (mean±SD change = -0.35±0.67). The YB treated dogs, however, demonstrated a 12.4% increase.

Study limitations stemmed from the logistics of the running of the JSAL. These labs involve many individuals performing different roles which creates variability. The admission and recruitment of patients for the study went well, however, the short time allotted to obtain the desired number of good surgical seropositive candidates was

Table 3: Individual and group coagulation parameters for Control Group dogs at baseline (Tuesday afternoon prior to surgery) and after 3 placebo doses (Tuesday pm, Wednesday am, Wednesday pm) on surgery morning post anesthetic induction (Thursday morning).

Dog Number	**PT** Baseline	Surgery am	**aPTT** Baseline	Surgery am	**Fibrinogen** Baseline	Surgery am	**Blood Loss** During Surgery
C/1	16.5	15.7	90.4	95.8	3.2	3.3	1.1
C/2	16.5	16.2	92.7	103.4	3.4	2.9	18.4
C/3	18.5	17.2	92.9	87.3	2.3	2.2	4.7
C/4	17.0	16.4	89.9	92.7	7.4	5.8	3.7
C/5	18.6	17.1	94.5	94.8	2.3	2.0	1.0
C/6	17.3	16.4	85.0	92.3	3.1	3.4	10.7
Group Mean±SD	17.4±0.94	16.5±0.57	90.9±3.35	94.4±5.31	3.62±1.91	3.27±1.36	6.60±6.78

Table 4: Individual and group coagulation parameters for Treatment Groups dogs at baseline (Tuesday afternoon prior to surgery) and after 3 doses of *Yunnan Baiyao* on surgery morning post anesthetic induction (Thursday morning).

Dog Number	**PT** Baseline	Surgery am	**aPTT** Baseline	Surgery am	**Fibrinogen** Baseline	Surgery am	**Blood Loss** During Surgery
T/1	17.2	16.8	85.2	80.0	3.9	3.9	2.4
T/2	16.2	17.4	99.3	99.0	3.2	3.6	4.1
T/3	17.4	16.5	89.4	95.6	2.2	2.6	3.9
T/4	17.0	17.0	84.2	85.7	1.7	1.9	11.9
T/5	17.1	17.3	77.2	87.8	2.5	2.9	9.1
T/6	18.0	17.1	104.3	89.2	2.0	2.5	7.2
Group Mean±SD	17.2±0.59	17.0±0.33	89.9±10.1	89.6±6.86	2.58±0.82	2.90±0.74	6.43±3.63

Table 5: Summary statistics on PT, aPPT, Fibrinogen, and Blood loss outcomes

		Pre-treatment (mean±SD)	**Post-treatment** (mean±SD)	**Change** (%; mean±SD; p-value)
PT	Control	17.4±0.94	16.5±0.57	-5.17%; -0.90±0.44; $p = 0.031$
	Treatment	17.2±0.59	17.0±0.33	-0.76%; -0.13±0.79; $p = 0.750$
aPTT	Control	90.9±3.35	94.4±5.31	+3.83%; +3.48±5.72; $p = 0.219$
	Treatment	89.9±10.1	89.6±6.86	-0.42%; -0.38±9.02; $p = 1.000$
Fibrinogen	Control	3.62±1.91	3.27±1.36	-9.67%; -0.35±0.67; $p = 0.344$
	Treatment	2.58±0.82	2.90±0.74	+12.4%; +0.32±0.18; $p = 0.063$
Blood Loss	Control		6.60±6.78	
	Treatment		6.43±3.63	

problematic. Any patient who arrived after Tuesday evening would not receive the three doses of placebo or YB and therefore was ineligible for the study. This directly affected the number of patients recruited to fill study groups within the time frame for data collection. In addition, the logistics to obtain blood samples before premedication of patients were not ideal at the time of data collection. Consequently, the post-dosing blood sample collection for evaluating the coagulation parameters had to be performed the morning of surgery after the dogs had been premedicated. The results might have been affected by unknown effects any of the premedication drugs may have on the mechanism of action of YB. To minimize the impact of this limitation, all blood samples were collected on both groups of dogs after premedication and processed in the same manner.

The reduced blood loss trend (3% of body weight) associated with YB treatment was small and did not achieve statistical significance in this study. The outcome of estimated blood loss may have been affected by the variation among students and instructors (a total of 4 students and 1 instructor per patient) participating in the surgical procedure. The subjective nature of estimating blood loss among different individuals by adding the number of used gauze sponges during the procedure and estimating the gauze blood saturation is not exact and probably prone to estimation error. This method also did not include the blood loss from surgical quarter/over drapes, surgical instruments/tray and surgeon gowns/gloves.

Taking into consideration the study limitations defined by this pilot study, a new clinical trial protocol is proposed. The clinical trial will span from 6 months to 1 year to ensure that a larger sample size is successfully attained. Oral administration of YB doses of 0.5g/5kg every twelve hours with the last dose before the surgery is recommended. Not dosing the study dogs on the morning of the surgery very likely diluted beneficial effects associated with YB administration as it has been shown that the herbal medicine has its greatest effects 3 hours after administration.[14] The premedication factor will be removed, and sample collection will occur upon admission to the lab, and after the surgical procedure. Additionally, a more objective method of estimating blood loss may involve weighing the gauze sponges used for hemostasis during surgery and comparing to the same number of clean gauze sponges, a modified method of estimating blood loss used in Liu's study.[12] A CBC and coagulation tests (buccal mucosal bleeding time, aPTT, PT, fibrinogen) will be collected upon admission and after the surgical procedure. Attention to platelet numbers will be emphasized since excessive bleeding due to coagulopathies (i.e., thrombocytopenia) is a concern in *E. canis* and *A. platys* seropositive dogs.[18]

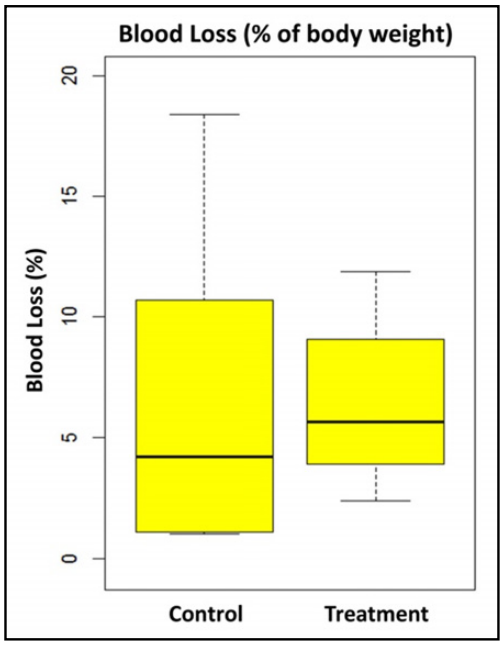

Figure 4: Distribution of blood loss in each of the study groups.

Based on evidence from the literature and this study, more robust studies are necessary to establish the effectiveness of preoperative oral administration of YB on tick-borne disease seropositive patient hemostasis. Important goals for these studies should include evaluating its effect on blood loss, coagulation parameters, platelet numbers and YB's anti-inflammatory properties. Finally, it will be important to establish an effective dose and timing for administration whereby this Chinese herbal medicine can be optimized for its intended purpose.

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Conflict of Interest and Funding

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FOOTNOTES

a. SNAP 4Dx© Plus Test. IDEXX, Westbrook, ME USA
b. *Yunnan Baiyao*, 0.25g capsules-16-pack. Jing Tang Herbal, Ocala, FL USA
c. Greenies Pill Pockets, The Nutro Company, Franklin, TN USA
d. 3cc-22g syringe-needle combination, Bectin Dickenson (BD) & Co. Franklin Lakes, TN USA
e. VetScan® VSPro Coagulation Analyzer, Zoetis, Parsippany-Troy, NJ USA
f. R (version 3.5.2; 2018-12-20), The R Foundation for Statistical Computing, Vienna Austria

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