The effects of oral administration of Yunnan Baiyao on blood coagulation in beagle dogs as measured by kaolin-activated thromboelastography and buccal mucosal bleeding times

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Abstract

We examined the effects of oral administration of Yunnan Baiyao (YB) on hemostasis by measuring buccal mucosal bleeding times (BMBTs) and doing citrated kaolin-activated whole-blood thromboelastography (TEG). In a randomized controlled crossover trial 8 beagle dogs were given either placebo or 1000 mg of YB orally every 12 h for 5 consecutive treatments. Blood was drawn 24 h before treatment and 2 and 24 h after the last treatment, and the BMBT was measured in each sample in duplicate. The TEG analysis was done in duplicate 60 ± 5 min after sample collection. There were no adverse effects of treatment and no significant differences between the control and treatment BMBTs or TEG parameters at any time point. Significant differences were found between baseline and 24 h after the last treatment within the treatment group for the TEG parameters LY30 and LY60 and within the control group for the TEG parameters MA, G, LY30, and LY60. Thus, at the dose and frequency of administration in this study YB did not appear to have any clinically significant effects on the measured coagulation parameters. The differences within the treatment group were likely due to analytic error since similar differences were seen in the control group. Further studies with a larger sample, as well as more direct measures of platelet function, are needed.

Résumé

Nous avons examiné les effets de l'administration orale de Yunnan Baiyao (YB) sur l'hémostase en mesurant le temps de saignement de la muqueuse buccale (TSMB) et en faisant une thromboélastographie (TEG) de sang entier après activation par de la kaoline citratée. Lors d'un essai en croisé randomisé et contrôlé, huit chiens beagle ont reçu soit un placebo ou 1000 mg de YB par voie orale à chaque 12 h pour cinq traitements consécutifs. Du sang a été prélevé 24 h avant le traitement et 2 et 24 h après le dernier traitement, et le TSMB mesuré dans chaque échantillon en duplicata. L'analyse TEG a été faite en duplicata 60 ± 5 min après le prélèvement de l'échantillon. Il n'y eut aucun effet néfaste du traitement et aucune différence significative entre le groupe témoin et le groupe traité pour ce qui est des TSMBs ou des paramètres de la TEG à tous les points d'échantillonnage. Des différences significatives ont été trouvées entre les valeurs de base et 24 h après le dernier traitement à l'intérieur du groupe traité pour les paramètres LY30 et LY60 de la TEG et à l'intérieur du groupe témoin pour les paramètres MA, G, LY30 et LY60 de la TEG. Ainsi, à la dose et à la fréquence d'administration utilisées dans la présente étude, YB ne semble pas avoir d'effet clinique significatif sur les paramètres de coagulation mesurés. Les différences dans le groupe traité sont fort probablement dues à une erreur analytique car des différences similaires ont été notées dans le groupe témoin. Des études supplémentaires avec un échantillonnage plus grand, ainsi que des mesures plus directes de la fonction des plaquettes sont requises.

(Traduit par Docteur Serge Messier)

Introduction

Yunnan Baiyao (YB) is a Chinese herbal supplement that has been used for its supposed hemostatic properties for almost a century. Knowledge of its specific properties is limited, but that has not deterred many physicians and veterinarians from employing it in various hemorrhagic and pathological conditions (1–4). The use of YB in canine veterinary practice is already widespread despite there being no reports of studies examining the efficacy of YB in reducing the incidence of hemorrhage in dogs.

The first studies that attempted to determine the efficacy of YB used laboratory animals as models. Topical administration of YB was shown to decrease bleeding times in rats and blood clotting times in rabbits (5,6). Another study showed that orally administered YB could shorten *in-vivo* bleeding time in rats when a piece of liver was excised and *in-vitro* blood clotting time in rabbits (7). It was also shown that YB could induce ultrastructural changes in platelets and platelet-constituent release, which possibly accounted for its hemostatic effects (8). Some later studies using humans as subjects determined that YB significantly reduced perioperative bleeding as well as bleeding in various ulcerative–hemorrhagic conditions (1,3,4).

Prior studies have found *in-vivo* bleeding times, such as those produced with liver laceration or tail transection, and template bleeding time (TBT) to be the most useful measures of YB efficacy (1,5–7,9). The results of other common coagulation tests, such as prothrombin time (PT), activated partial thromboplastin time (aPTT), and the d-dimer test, were not affected in human patients or in dogs treated with YB (1,10). The activated clotting time (ACT) was also not affected in ponies treated with YB (9). Citrated kaolin-activated whole-blood thromboelastography (TEG) has only once before been

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used to measure the efficacy of YB, with no significant results, but may be useful because of its ability to assess all coagulation parameters, including fibrinolysis (10).

The objective of this study was to determine the effects of oral administration of YB on coagulation in 8 beagle dogs as measured by TEG and buccal mucosal bleeding times (BMBTs). Our hypothesis was that this treatment would result in TEG tracings with decreased R and K values and decreased lytic parameters, as well as a shortened BMBT as compared with control treatment. The R value represents the reaction time; that is, the time until the first evidence of a clot is detected. The K value is the time from the end of R until the clot reaches 20 mm in diameter; this represents the speed of clot formation.

Materials and methods

Eight university-owned beagle dogs (5 male and 3 female) were used in this study. Inclusion criteria included normal results of a physical examination, a normal initial complete blood (cell) count (CBC) and biochemistry panel, and a normal coagulation profile as measured by BMBT and TEG analysis. The dogs were between 5 and 8 y old, and their mean body weight (BW) was 10.7 ± 1.5 kg (extremes 8.9 and 12.8 kg). Two of the dogs received cyclosporine ophthalmic ointment daily throughout the study for treatment of chronic keratoconjunctivitis sicca. Animal care protocols were approved by the University of Calgary Veterinary Sciences Animal Care Committee (VSACC). Protocol number AC13-0266

The dogs were individually housed in runs overnight and then spent about 8 h in group outdoor yards. The 3 youngest dogs spent outdoor time together, as did the 5 oldest. Seven of the dogs were fed a standard commercial dog food twice per day, whereas the dog that was overweight was fed a low-calorie commercial dog food twice per day.

The dogs were randomly assigned to 2 groups: YB and control. The researchers were blind to the group assignments until after data analysis. The YB group was treated with 4 × 250 mg (1000 mg) of YB divided equally into 2 Greenies Pill Pockets [Nutro Products (a subsidiary of Mars Incorporated), Franklin, Tennessee, USA]. The control group received 2 empty Pill Pockets. Treatment was every 12 h for 5 doses. For ease of dosing and blood sampling the dogs were managed in pairs, 2 h apart.

Blood was collected 24 h before the first treatment (baseline) and 2 h and 24 h after the last treatment. With the use of 20-gauge 1-inch Monoject needles and 6-mL Monoject syringes (Medtronic, Minneapolis, Minnesota, USA) 2 mL of blood was collected from alternating jugular veins; that is, if the first attempt to obtain a blood sample failed, then the contralateral jugular vein was used. The blood was immediately transferred into sterile BD Vacutainer tubes containing 3.2% sodium citrate (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA). The tubes were filled to the maximum fill line to ensure a 9:1 ratio of blood to anticoagulant, were inverted 5 times to allow mixing of blood and anticoagulant, and were kept at room temperature until TEG analysis was started, 60 ± 5 min after collection.

The BMBT was measured 24 h before the first treatment (baseline) and 2 and 24 h after the last treatment. The lancets used to create a small, standardized wound in the buccal mucosa were single-use disposable devices (Surgicutt; International Technidyne Corporation, Piscataway, New Jersey, USA). Three people were present for each test, one to restrain the dog in lateral recumbency, one to hold the lip up as well as to start and stop a timer, and one to use the lancet. In one case the dog's excessive head movement necessitated securing the lip with a piece of roll gauze. The buccal mucosa was dried with a towel if excessive saliva was present. The timer was started when the lancet was used. Blotting paper was used to blot blood as it accumulated at the periphery of the incision while avoiding the incision itself to prevent disruption of the clot. The timer was stopped when the blotting paper no longer absorbed blood from the periphery. The incision was repeated on the other side of the mouth, and the average of the 2 times was recorded for each dog.

A 10-day washout period was allowed, after which a crossover study was done in the same manner.

The TEG analysis was done at 37°C in duplicate simultaneously on the same Thromboelastograph Hemostasis Analyzer (Haemonetics, Braintree, Massachusetts, USA). The average of the 2 values was used for statistical analysis. In brief, 1 mL of citrated whole blood was transferred to room-temperature vials containing 40 μL of kaolin. The tubes were inverted gently 5 times to allow mixing of blood and activator; 340 μL of this mixture was pipetted into TEG cups containing 20 μL of room-temperature 0.2 M calcium chloride, and the TEG analysis was started. The TEG analyses were run until the R and K values, the angle between R and K (α-angle), the maximum amplitude (MA), the shear elastic modulus strength (G), and the percentage clot lysis at 30 and 60 min (LY30 and LY60) were finalized. Electronic tests along with level I (normal tracing) and level II (hypocoagulable tracing) control tests were run every morning of analysis to ensure accurate results.

Statistical analysis

All results were tested for normalcy with the d'Agostino and Pearson Omnibus Normality Test. For all data sets that passed this test, within-group comparisons were done by 1-way repeated-measures analysis of variance (ANOVA) with a Dunnett's multiple-comparisons test. For all data sets that failed to pass the normalcy test, a repeated-measures Friedman's test was used with a *post-hoc* Dunn's multiple-comparisons test. For between-groups comparisons a 2-way ANOVA and *post-hoc* Bonferoni test were used.

Results

Both the initial and crossover phases of the trial were completed with all 8 dogs. No adverse effects were noted throughout the study according to the results of physical examination. All TEG controls had results within the reference ranges throughout the study. All data passed the normalcy test with the exception of R and α-angle.

There were no significant between-group differences in the means of any of the parameters measured in this study. The 95% confidence intervals (CIs) of the mean differences (Table I) demonstrated a large range of values, and all included the zero-difference value.

In the control group there was a significant within-group difference between the mean values for baseline and 24-hour MA ($P = 0.04$) and G ($P = 0.03$); the *post-hoc* Dunnett's test showed means of 56.08 ± 4.517 (standard deviation) and 6.513 ± 1.113, respectively,

Table I. Mean differences in buccal mucosal bleeding time (BMBT) and thromboelastography parameters in 8 dogs before and after treatment with Yunnan Baiyao and before and after a control period during a randomized controlled crossover study

Time or parameter	Difference between groups	Time in relation to treatment		
		24 h before	2 h after	24 h after
BMBT (s)	Mean	8.88	8.38	−10.5
	95% CI	−24.85 to 42.6	−25.35 to 42.1	−44.22 to 23.22
	P-value	> 0.99	> 0.99	> 0.99
R (min)	Mean	0.32	−0.58	−0.13
	95% CI	−0.7 to 1.33	−1.6 to 0.43	−1.14 to 0.9
	P-value	> 0.99	0.48	> 0.99
α-angle (°)	Mean	2.18	1.78	−1.54
	95% CI	−3.46 to 7.8	−3.85 to 7.41	−7.18 to 4.09
	P-value	> 0.99	> 0.99	> 0.99
K (min)	Mean	−0.21	−0.21	0.09
	95% CI	−0.76 to 0.35	−0.76 to 0.35	−0.47 to 0.64
	P-value	> 0.99	> 0.99	> 0.99
MA (mm)	Mean	0.7	−1.73	−2.93
	95% CI	−4.48 to 5.88	−6.91 to 3.45	−8.11 to 2.25
	P-value	> 0.99	> 0.99	0.5
G (dynes/s)	Mean	0.21	−0.37	−0.9
	95% CI	−1.09 to 1.5	−1.66 to 0.92	−2.19 to 0.39
	P-value	> 0.99	> 0.99	0.27
LY30 (%)	Mean	0.15	0.57	0.9
	95% CI	−5.21 to 5.51	−4.79 to 5.93	−4.46 to 6.26
	P-value	> 0.99	> 0.99	> 0.99
LY60 (%)	Mean	1.21	1.67	0.44
	95% CI	−6.87 to 9.28	−6.4 to 9.74	−7.63 to 8.51
	P-value	> 0.99	> 0.99	> 0.99

CI — confidence interval; R — reaction time; α-angle — angle between R and K; K — time from 2 to 20 mm in amplitude; MA — maximum amplitude; G — shear elastic modulus strength; LY30 — percent clot lysis at 30 min; LY60 — percent clot lysis at 60 min.

at baseline and 52.93 ± 2.473 and 5.663 ± 0.5662, respectively, at 24 h ($P = 0.05$ for both). The control group also showed a significant within-group difference between the mean values for baseline and 24-hour LY30 ($P < 0.01$) and LY60 ($P = 0.02$); the *post-hoc* Dunnett's test showed means of 2.369 ± 3.625 and 6.719 ± 7.334, respectively, at baseline and 7.438 ± 3.744 and 12.79 ± 4.714, respectively, at 24 h ($P < 0.05$ for both).

In the YB group there was a significant within-group difference between the mean values for baseline and 24-hour LY30 ($P = 0.03$) and LY60 ($P = 0.02$, respectively); the *post-hoc* Dunnett's test showed means of 2.219 ± 3.148 and 5.513 ± 5.507 at baseline and 6.538 ± 5.422 and 12.35 ± 6.058 at 24 h ($P < 0.05$ for both).

There were no significant within-group differences in BMBT, R, K, or α-angle.

Discussion

This study aimed to obtain more information on how YB affects coagulation in canines through the use of *in-vivo* and *in-vitro* methods. We ultimately chose to use the BMBT to evaluate *in-vivo* bleeding times because many earlier studies used similar *in-vivo* bleeding times, such as liver and tail transection and TBT, and they reported significant differences between treatment and control groups (5–7,9). However, in our study there was no significant difference between treatment and control groups in BMBT or in the TEG values. The 95% CI of the mean between-group differences revealed a large range of values that included zero difference for all the parameters. These collective results suggest that YB, at the dose and frequency of administration that we used, did not have a measurable effect on coagulation in the 8 dogs studied.

There were significant within-group differences between baseline and 24 h for the LY30 and LY60 TEG values in our study; however, the fact that these changes occurred in both the control and treatment groups argues against the differences being due to the administration of YB. There were no significant within-group changes in BMBT.

The results of this study do not correlate with those in the current, limited YB literature, in which reported studies in both animals and humans have yielded results suggesting that YB has a measurable

effect on coagulation, particularly on bleeding times (1,4–7,9). It is uncertain why in this study the dogs did not demonstrate any observable differences, but this could be due to observer error, patient-related factors, product variation, dosage inadequacy, species differences, or small sample size.

The BMBT has been shown to be a useful *in-vivo* test of primary hemostasis, but multiple studies have shown that there is great interobserver and intraobserver variation in BMBTs recorded on the same animal (11,12). To try to minimize variation in this study, the same observer conducted all BMBT tests on all animals. However, some intraobserver variability could have been partially responsible for the statistical insignificance of the results. This could have been due to the relative inexperience of the observer, although the experimenter had practised on 2 dogs to refine the technique. The observer was blinded to treatment assignments until after data analysis to eliminate any bias when recording times.

Animal-related factors could have played a role in the variability of measurements as well. There were times when dogs struggled against the restraint, which could have led to increased blood pressure and subsequent disruption of the forming clot. However, there were always 3 people assisting to minimize patient-behavior factors.

Variability can be seen when different-sized BMBT devices are used (13). In the present study all BMBT devices were the same size throughout the study to minimize obvious variation of incision size. We chose the manufacturer-recommended device size for use in patients of this weight category.

Another reason for the lack of difference between groups may be related to the lack of quality control in the production of YB. This nutraceutical is unregulated and therefore could vary from batch to batch. Owing to logistical difficulties we were forced to use YB bought at different times from different sources. It could have been manufactured at very different times, and the ingredients and amounts could have varied in a manner that rendered some of the batches less efficacious.

The package insert for the YB used recommends oral administration of 1 to 2 of the 250-mg capsules up to 4 times per day in humans. There are no specific dosage recommendations published for animals. The dosage used in this study (1000 mg per 5- to 15-kg dog twice daily) was based on the results of a previous clinical study in dogs (10) and our clinical experience that most practitioners use 2 to 3 times the manufacturer-recommended dose. A study in rats yielded significant results when the dose was 240 mg/kg, much higher than that used in this study (7). It is possible, therefore, that the dosage we chose was too low for an effect to be seen. Without prior studies evaluating the efficacy of oral YB treatment on hemostasis in dogs, the administration and sampling times in this study were based on trials in humans and other animals that observed clinical effects of oral YB treatment. In rabbits and rats a significant hemostatic effect was observed according to the BMBT starting 30 min after administration of YB and persisting till at least 4 h after administration (7). The duration of efficacy of orally administered YB in dogs has not yet been established, and extrapolation from other species may have led to sampling times in our study that did not reflect peak hemostatic effects.

Another important consideration is that it is not known if there are species variations in the efficacy of YB. It is possible that canines do not respond in the same way as humans and small rodents. Further studies are needed in domestic animals to determine whether there are species differences in the response to YB.

An important limitation of the present study is the relatively small sample size. With only 8 dogs, the power in this study was limited and the range of values representing the difference of the means between groups quite large. Clinical trials with larger samples might be able to detect smaller differences between groups and provide a narrower range of values wherein the true mean difference between groups would lie. However, the clinical importance of these ranges of mean differences in TEG parameters remains difficult to fully interpret.

Another consideration is the fact that 2 of the dogs used in this study were receiving topical cyclosporine treatment of chronic keratoconjunctivitis sicca. Systemic administration of cyclosporine can induce a hypercoagulable state in humans (14). Oral administration of cyclosporine to dogs has been shown to alter the platelet membrane and is associated with a significant increase in thromboxane production (15). There are no published reports of studies that showed that topical administration yielded the same effect in canines. The results of the present study do not seem to indicate that the 2 dogs that were given cyclosporine had any significant changes in their coagulation parameters as compared with the other dogs. The TEG tracings for these 2 dogs indicated normal coagulation, and the BMBTs were comparable to those of the other dogs, which suggests that the topical application of cyclosporine was not responsible for a hypercoagulable state in these dogs.

The significant differences in the TEG values in both the control and treatment groups do not appear to be due to the administration of YB, as within-group significant differences in LY30 and LY60 were present in both groups. There could be multiple explanations as to why these differences occurred. The TEG machines are extremely sensitive to preanalytic and analytic factors (16). Even though measures were taken to decrease the presence of these factors, such as using a standardized sample-collection technique, having a single person collecting and analyzing all the samples, and using standardized temperatures of analyses, there still could have been some variation in preanalytic and analytic techniques that led to these differences. The TEG analysis for all samples was done at 37°C, as the reference intervals for kaolin-activated canine samples were determined at this temperature (17). Because the physiological temperature of the healthy canine is normally between 38°C and 39°C, there may have been some differences in clotting kinetics with the lower temperature of analysis. This temperature was chosen for ease of comparison with reference intervals.

A recent study in dogs found that TEG was not a sensitive indicator of hypercoagulable states and suggested that a shortened PT/PTT maybe a more sensitive indicator in some situations (17). It is possible that the dogs used in our study in fact had hypercoagulable states but the tests used were unable to detect this. However, a study investigating oral YB administration in dogs at a lower dose than used in the current study failed to show a significant difference in PT, aPTT, fibrinogen, and d-dimer values when compared with the values in control dogs (10).

Reference intervals have been established for kaolin-activated samples in canines (18). All of the mean values in the present study fell within these intervals with the exception of those for LY30 and

LY60, for which reference intervals were not available for canine patients. Although the within-group differences for MA and G were significant, they fell within these established reference ranges. Thus, the differences in MA and G in the control group were likely of no clinical significance and could have been due to preanalytic and/or analytic variation as discussed.

In the present study, financial and time constraints limited platelet function analysis to the use of BMBT. Further studies could include more direct measures of platelet function. It has been determined that YB can cause the release of platelet constituents and other ultrastructural changes (8). It would have been interesting to see under the electron microscope how canine platelets respond to YB. Other tests that might have helped to determine the nature of YB's effect on platelets could have included TEG platelet mapping, as this technique has been shown to detect hypercoagulable states in canines (19). Another useful test would have been platelet aggregometry, as this has been found to be useful in testing platelet function in dogs (20).

At the dosage and frequency of administration used in this study, YB seems to be safe for dogs. Physical examination yielded no change in results from baseline. It is unknown, however, if YB has any effect on blood parameters that are routinely measured, as these were assessed only in the initial phase, before dosing. More in-depth studies are needed to confirm the safety of the product in terms of the complete blood count and biochemical parameters after dosing.

In conclusion, Yunnan Baiyao, at the dosage used in this study (1000 mg per 5- to 15-kg dog twice a day), given orally for 5 consecutive treatments, appears to be safe for canines. It did not produce a significant reduction in BMBT or changes in TEG results, including the R and K values and fibrinolysis, in a comparison of the control and YB groups.

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References

1. Tang ZL, Wang X, Yi B, Li ZL, Liang C, Wang XX. Effects of the preoperative administration of Yunnan Baiyao capsules on intraoperative blood loss in bimaxillary orthognathic surgery: A prospective, randomized, double-blind, placebo-controlled study. Int J Oral Maxillofac Surg 2009;38:261–266. Epub 2009 Jan 18.
2. Salgado B, Paramo R, Sumano H. Successful treatment of canine open cervix–pyometra with yun-nan-pai-yao, a Chinese herbal preparation. Vet Res Commun 2007;31:405–412.
3. Li R, Alex P, Ye M, Zhang T, Liu L, Li X. An old herbal medicine with a potentially new therapeutic application in inflammatory bowel disease. Int J Clin Exp Med 2011;4:309–319. Epub 2011 Oct 29.
4. Yang B, Xu Z, Zhang H, et al. The efficacy of Yunnan Baiyao on haemostasis and antiulcer: A systematic review and meta-analysis of randomized controlled trials. Int J Clin Exp Med 2014; 7:461–482.
5. Ogle CW, Dai S, Ma JC. The haemostatic effects of the Chinese herbal drug Yunnan bai yao: A pilot study. Am J Chin Med (Gard City N Y) 1976;4:147–152.
6. Fan C, Song J, White CM. A comparison of the hemostatic effects of notoginseng and yun nan baiyao to placebo control. J Herb Pharmacother 2005;5:1–5.
7. Ogle CW, Dai S, Cho CH. The hemostatic effects of orally administered Yunnan bai yao in rats and rabbits. Comp Med East West 1977;5:155–160.
8. Chew EC. Effects of Yunnan bai yao on blood platelets: An ultrastructural study. Comp Med East West 1977;5:169–175.
9. Graham L, Farnsworth K, Cary J. The effect of Yunnan bai yao on the template bleeding time and activated clotting time in healthy halothane anesthetized ponies [abstract]. In: Proceedings of the International Veterinary Emergency and Critical Care Symposium, 2002, San Antonio, Texas. J Vet Emerg Crit Care (San Antonio) 2002;12:279.
10. Lee A, Boysen S, Chalhoub S, Sanderson J, Wagg C. Effects of Yunnan Baiyao on blood coagulation parameters in beagles measured using kaolin activated thromboelastography [abstract]. J Vet Emerg Crit Care 2015;25(S1):S24 doi: 10.1111/vec.12366
11. Alatzas DG, Mylonakis ME, Kazakos GM, Kostoulas P, Kritsep-Konstantinou M, Polizopoulou ZS. Reference values and repeatability of buccal mucosal bleeding time in healthy sedated cats. J Feline Med Surg 2014;16:144–148. Epub 2013 Aug 28.
12. Sato I, Anderson GA, Parry BW. An interobserver and intraobserver study of buccal mucosal bleeding time in Greyhounds. Res Vet Sci 2000;68:41–45.
13. Aumann M, Rossi V, Le Boedec K, Diquelou A. Comparison of the buccal mucosal bleeding time in dogs using 3 different-sized lancet devices. Vet Clin Pathol 2013;42:451–457.
14. Tomasiak M, Rusak T, Gacko M, Stelmach H. Cyclosporine enhances platelet procoagulant activity. Nephrol Dial Transplant 2007;22:1750–1756.
15. Thomason J, Lunsford K, Stokes J, et al. The effects of cyclosporine on platelet function and cyclooxygenase expression in normal dogs. J Vet Intern Med 2012;26:1389–1401. Epub 2012 Oct 28.
16. Walker JM, Hanel RM, Hansen BD, Motsinger-Reif AA. Comparison of venous sampling methods for thromboelastography in clinically normal dogs. Am J Vet Res 2012;73:1864–1870.
17. Bauer N, Eralp O, Moritz A. Establishment of reference intervals for kaolin-activated thromboelastography in dogs including an assessment of the effects of sex and anticoagulant use. J Vet Diagn Invest 2009;21:641–648.
18. Song J, Drobatz KJ, Silverstein DC. Retrospective evaluation of shortened prothrombin time or activated partial thromboplastin time for the diagnosis of hypercoagulability in dogs: 25 cases (2006–2011). J Vet Emerg Crit Care (San Antonio) 2016;26:398–405.
19. Park FM, Blois SL, Abrams-Ogg AC, et al. Hypercoagulability and ACTH-dependent hyperadrenocorticism in dogs. J Vet Intern Med 2013;27:1136–1142. Epub 2013 Aug 26.
20. Marschner CB, Kristensen AT, Spodsberg EH, Wiinberg B. Evaluation of platelet aggregometry in dogs using the Multiplate platelet analyzer: Impact of anticoagulant choice and assay duration. J Vet Emerg Crit Care (San Antonio) 2012;22:107–115.