

# Effects of doxycycline, amoxicillin, cephalixin, and enrofloxacin on hemostasis in healthy dogs

Jinelle A. Webb, DVM, DVSc; Dana G. Allen, DVM, MSc; Anthony C. G. Abrams-Ogg, DVM, DVSc; Patricia A. Gentry, PhD

**Objective**—To determine the effects of enteral administration of doxycycline, amoxicillin, cephalixin, and enrofloxacin at therapeutic dosages for a typical duration on hemostatic variables in healthy dogs.

**Animals**—14 Beagles.

**Procedure**—Doxycycline (10 mg/kg, PO, q 12 h), amoxicillin (30 mg/kg, PO, q 12 h), cephalixin (30 mg/kg, PO, q 12 h), and enrofloxacin (20 mg/kg, PO, q 24 h) were administered in random order to 10 healthy dogs at standard therapeutic dosages for 7 days, with a 7-day washout period between subsequent antimicrobials. In addition, 4 Beagles served as control dogs. Variables were evaluated before and after antimicrobial administration; they included platelet count, Hct, 1-stage prothrombin time (PT), activated partial thromboplastin time (PTT), fibrinogen concentration, and platelet function. Platelet function was assessed via buccal mucosal bleeding time, aggregation, and a platelet-function analyzer.

**Results**—Administration of all antimicrobials caused a slight prolongation of 1-stage PT and activated PTT and slight decrease in fibrinogen concentration. Cephalixin caused a significant increase in 1-stage PT and activated PTT, amoxicillin caused a significant increase in activated PTT, and enrofloxacin caused a significant decrease in fibrinogen concentration. Platelet count or function did not differ significantly after administration of any antimicrobial.

**Conclusions and Clinical Relevance**—Oral administration of commonly used antimicrobials in healthy dogs resulted in minor secondary hemostatic abnormalities, with no change in platelet count or function. Although these changes were clinically irrelevant in healthy dogs, additional studies of the effects of antimicrobial administration on hemostasis in animals with underlying disease processes are warranted. (*Am J Vet Res* 2006;67:xxx-xxx)

Primary hemostatic disturbances are common in dogs.<sup>1</sup> The most common primary hemostatic disturbance is thrombocytopenia; however, thrombocy-

## ABBREVIATIONS

BMBT	Buccal mucosal bleeding time
PT	Prothrombin time
PTT	Partial thromboplastin time
PRP	Platelet-rich plasma
PPP	Platelet-poor plasma
ADP	Adenosine diphosphate
PAF	Platelet-activating factor
NMTT	N-methyl-thio-tetrazole

topathia is probably underdiagnosed because of the difficulty in assessing platelet function.<sup>1,2</sup> There are several acquired causes of platelet dysfunction, including uremia, hepatic disease, disorders affecting megakaryocytes (chronic myeloproliferative disorders, leukemia, and myelodysplastic syndromes), and dysproteinemia.<sup>1</sup> Platelet dysfunction may also be a component of immune-mediated thrombocytopenia.<sup>3</sup>

Secondary hemostasis is required to form a stable secondary platelet plug. Hemostasis has traditionally been divided into the extrinsic and intrinsic system, but consideration is being given to revise this division to reflect a system that more accurately reflects the physiologic processes of hemostasis.<sup>4</sup> Secondary hemostatic disturbances can be detected at the time of initial examination and by use of laboratory testing in dogs, and abnormalities include inherited and acquired forms.<sup>4</sup>

Many drugs reportedly cause thrombocytopenia, including chemotherapeutic agents, antiviral drugs, anticonvulsants, anti-inflammatory drugs, estrogenic compounds, many antimicrobials, and cardiovascular drugs.<sup>1,5</sup> There are also several drugs that can cause platelet dysfunction, such as cyclooxygenase inhibitors, antimicrobials, synthetic colloids, barbiturates, antihistamines, several chemotherapeutic agents, and selective serotonin-reuptake inhibitors.<sup>1,5</sup> Drugs that commonly cause acquired abnormalities of secondary hemostasis include heparin and vitamin K antagonists, such as warfarin.<sup>2</sup>

Antimicrobials are widely used in human and veterinary medicine. Their use ranges from prophylactic broad-based perioperative administration to targeted use against identified bacterial infections. Some classes of antimicrobials may be avoided because of assumptions about their possible effects on hemostasis. Studies<sup>6-8</sup> in humans and dogs indicate that certain antimicrobials cause a disturbance in primary hemostasis, including thrombocytopenia and platelet dysfunction. The most commonly implicated antimicrobials include those in the  $\beta$ -lactam family; however, disturbances associated with sulfonamides, tetracyclines, chloramphenicol, aminoglycosides, metronida-

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From the Departments of Clinical Studies (Webb, Allen, Abrams-Ogg) and Biomedical Sciences (Gentry), Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1, Canada. Dr. Webb's present address is Veterinary Emergency Clinic, 920 Yonge St, Toronto, ON M4W 3C7, Canada.

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Address correspondence to Dr. Webb.

zole, imipenem, and fluoroquinolones have also been identified in humans.<sup>5,6,9-12</sup> In dogs, only  $\beta$ -lactams, sulfonamides, tetracyclines, and chloramphenicol have been associated with platelet abnormalities.<sup>1,5,13</sup> There appear to be substantial differences among species with regard to the hemostatic abnormalities that result from antimicrobial administration. In other species that have been evaluated, such as cattle<sup>14</sup> and pigs,<sup>15</sup> platelet function is not affected by penicillins or metronidazole. Although secondary hemostasis is not commonly affected by antimicrobials, the cephalosporins appear to cause dysfunction in hemostasis by inducing hypoprothrombinemia.<sup>16,17</sup>

Investigators in studies<sup>6,18</sup> conducted to evaluate the effect of antimicrobials on primary and secondary hemostasis have often used dosages of antimicrobials far in excess of those necessary to achieve therapeutic concentrations. There have been extremely few *in vivo* studies<sup>8,19</sup> conducted to assess the effect of antimicrobials on hemostasis in domestic animals, and these have focused primarily on cephalosporins. The objective of the study reported here was to determine the effects of enteral administration of doxycycline, amoxicillin, cephalexin, and enrofloxacin at therapeutic dosages for a typical clinical duration on hemostatic variables in healthy dogs.

## Materials and Methods

**Animals**—Fourteen healthy sexually intact purposebred Beagles (7 males and 7 females) were used in the study. Dogs ranged from 10 to 18 months of age and weighed between 7.0 and 11.1 kg. All had received routine vaccinations against canine distemper virus, parvovirus, adenovirus types 1 and 2, parainfluenza virus, rabies virus, and *Bordetella bronchiseptica* at least 6 months, but not more than 12 months, before start of the study. Dogs were allowed to acclimate to the animal facility for at least 2 months prior to initiation of the study, and no dogs had clinical signs of disease during their acclimation period.

All dogs were confirmed to be healthy on the basis of results of physical examination, a CBC count, serum biochemical analysis, and urinalysis conducted within 1 week before initiation of the study. Primary and secondary hemostasis were determined to be within expected limits for healthy dogs as determined on the basis of a platelet count, BMBT, closure time measured by use of a platelet-function analyzer,<sup>3</sup> platelet aggregation, 1-stage PT, and activated PTT. Closure time was defined as the time required for platelets to seal a 147- $\mu$ m aperture in a membrane coated in equine collagen and ADP and therefore stop blood flow in the platelet function analyzer. Because Beagles can be deficient in factor VII,<sup>2</sup> activity of this factor was measured and was within the reference range in all dogs.

Dogs were housed indoors, fed a standard commercially available kibble formulated for dogs, and allowed to walk outside in a restricted area on a leash twice weekly. This study was designed and conducted in accordance with standards of the Canadian Council on Animal Care and the Ontario Animals for Research Act and was approved by the University of Guelph Animal Care Committee.

**Study design**—Ten dogs were orally administered 4 antimicrobials at dosages consistent with current therapeutic recommendations.<sup>20</sup> The dogs were administered doxycycline<sup>b</sup> (10 mg/kg, PO, q 12 h), amoxicillin<sup>c</sup> (30 mg/kg, PO, q 12 h), cephalexin<sup>d</sup> (30 mg/kg, PO, q 12 h), and enrofloxacin<sup>e</sup> (20 mg/kg, PO, q 24 h). Each antimicrobial was administered for

7 days, and there was a 7-day washout period between subsequent treatments (a minimum of 14 half-lives for each drug). An incomplete Latin square randomization procedure was used to determine the order in which each dog received each antimicrobial to control for period and carryover effects. Antimicrobials were administered in liquid formulations to allow for accurate dosing. Antimicrobials were administered with food, with the exception of the antimicrobial administered on day 7.

Four dogs served as control animals. These dogs did not receive any treatments during an 8-week period (ie, study duration). The entire study was completed within a 14-month period.

Hemostatic testing was conducted on the day of initiation of each treatment (baseline) and within 2 hours after each dog was administered the final dose of an antimicrobial on day 7 of each treatment. Food was withheld for a minimum of 12 hours before collection of blood samples for hemostatic testing. Investigators were not aware of the treatments administered to each dog.

**Collection of blood samples**—Venous blood samples were collected via puncture of the jugular vein by use of a 20-gauge, thin-walled needle. Volume of blood collected was determined on the basis of the amount needed for each dog to enable harvest of a sufficient amount of PRP. Blood samples were immediately transferred into siliconized 10-mL glass tubes; each tube contained 1 mL of 3.2% sodium citrate as anticoagulant and 9 mL of whole blood. All of the dogs were acclimated to blood collection procedures, and each dog allowed rapid, efficient collection of samples.

**Analysis of hemostasis**—Primary hemostatic variables measured in treated dogs included BMBT, closure time measured by use of a platelet-function analyzer,<sup>3</sup> and platelet aggregation. Secondary hemostatic variables measured included 1-stage PT; activated PTT; concentration of fibrinogen (factor I); and activities of factors II, V, VII, VIII, IX, and X. Other variables assessed included platelet count, Hct, and WBC count. Variables evaluated for the control dogs included those that were significantly changed after antimicrobial administration, which included 1-stage PT, activated PTT, and fibrinogen concentration. In addition, platelet count, Hct, and WBC count were also available.

**Platelet count, Hct, and WBC count**—Approximately 800  $\mu$ L of citrated whole blood was transferred to polyethylene microtubes. Platelet count, Hct, and WBC count were quantified by use of an automated hematologic counter.<sup>f</sup> Blood smears were also evaluated microscopically to assess platelet clumping.

**Bleeding time**—The BMBT was determined as described elsewhere.<sup>21</sup> Briefly, each dog was restrained in lateral recumbency and the maxillary lip was retracted and held in place with a strip of gauze. The gauze was tightened sufficiently to hold the lip in place without permitting substantial blood stasis. A template device<sup>g</sup> was placed on the buccal mucosa at a point approximately halfway between the mucocutaneous junction and gingiva. The device was triggered, which resulted in a small incision parallel to the lip margin. Blotting paper<sup>h</sup> was positioned adjacent to the incision to remove blood as it seeped from the incision. Disruption of the formation of a primary platelet plug can create erroneous results. Therefore, investigators were careful to ensure that the incision was not touched with the blotting paper; however, fibrin tags were removed as described elsewhere.<sup>22</sup> The BMBT was recorded as the interval from triggering of the device until blood ceased to flow from the incision.

**Platelet function as measured by use of a platelet-function analyzer**—Platelet function was assessed by use of a

platelet-function analyzer<sup>a</sup>; ADP was used as an agonist. The ADP cartridge was warmed to 22°C and then placed in the analyzer, which further warmed the samples to 37°C prior to measurement of platelet function. An 800- $\mu$ L aliquot of citrated whole blood was transferred to the cartridge by use of an adjustable piston micropipetter,<sup>i</sup> and closure time was recorded. Analysis was performed within 2 hours after collection of blood samples. The analysis was repeated on 5 samples because errors were reported by the analyzer.

**Platelet aggregation**—An aliquot of citrated whole blood was centrifuged (80  $\times$  g for 10 minutes). The supernatant (ie, PRP) was removed by use of a polyethylene pipette<sup>j</sup> and transferred to polystyrene tubes<sup>k</sup> that were sealed with caps. When there was an insufficient amount of PRP harvested, the process was repeated once with the remaining plasma. The PRP was gently mixed by manually rotating the tubes, and tubes were then allowed to sit undisturbed for 10 minutes. The remaining plasma was centrifuged (3,000  $\times$  g for 10 minutes), and the PPP was removed by use of a polyethylene pipette<sup>j</sup> and transferred to polystyrene tubes<sup>k</sup> that were sealed with caps.

A standard manual method for evaluation of platelet concentration was used to determine the platelet count in each sample of PRP.<sup>23</sup> A 20- $\mu$ L aliquot of PRP was added to a commercial dilution system for platelet determinations<sup>l</sup> (final dilution, 1:100). The solution was gently mixed manually and then allowed to sit undisturbed for 10 minutes. The 2 counting chambers of a hemacytometer<sup>m</sup> were filled with the dilute PRP solution, and the hemacytometer was then placed in a humidified Petri dish for 10 minutes. The chambers were viewed at 500 $\times$  magnification by use of light microscopy, and the number of platelets in the 25 main squares of each counting chamber was counted. The mean value was determined for the 2 chambers and multiplied by 1,000 to provide an estimate of the platelet count in PRP.

Appropriate quantities of PRP were mixed with autologous PPP in a plastic test tube to yield PRP with a final platelet count of 200  $\times$  10<sup>9</sup> platelets/L. This standardized PRP was gently mixed by rotating the tube manually, then the tube was sealed with a plastic cap. The mixture was left to sit undisturbed for a minimum of 30 minutes prior to aggregometry.

Platelet agonists used in the study included ADP<sup>n</sup> and PAF<sup>o</sup>, both of which were administered at a high and low concentration. The high concentration selected was the concentration that consistently induced maximum aggregation in all dogs, and the low concentration selected was the concentration that less consistently induced complete aggregation. The 2 concentrations of both agonists were prepared before initiation of aggregometry. The ADP was diluted in Tris-buffered saline solution (pH, 7.4) to achieve the desired concentrations. For most dogs, the concentrations of ADP solutions were 1  $\times$  10<sup>-4</sup>M (high concentration) and 5  $\times$  10<sup>-5</sup>M (low concentration). Two dogs required higher doses of ADP to cause substantial aggregation; therefore, concentrations of ADP solution were 5  $\times$  10<sup>-4</sup>M (high concentration) and 1  $\times$  10<sup>-4</sup>M (low concentration) for those dogs. Lower concentrations of PAF were required to cause substantial aggregation; therefore, PAF was dissolved in ethanol and then diluted in Tris-buffered saline solution (pH, 7.4) to achieve concentrations of 5  $\times$  10<sup>-7</sup>M (high concentration) and 1  $\times$  10<sup>-7</sup>M (low concentration). Platelets from all dogs aggregated well at these concentrations of PAF.

Platelet aggregation was measured within 4 hours after collection of blood samples. Platelet aggregation was measured optically by use of a dual-channel aggregometer.<sup>p</sup> Small magnetic stir bars were loaded into disposable, siliconized glass cuvettes. A 225- $\mu$ L aliquot of PRP or a 250- $\mu$ L aliquot of autologous PPP was added to each cuvette. Samples were

analyzed in duplicate (ie, 2 cuvettes with PRP and 2 cuvettes with PPP for each sample). All cuvettes were placed in the aggregometer, where they were warmed to 37°C. The plasma was constantly stirred at 900 rounds/min<sup>q</sup> to allow for adequate platelet distribution and rapid mixing of added agonists. Stirring was also used to maintain consistency of the PPP. Maximum transmission was determined by measuring the transmission of light through the PPP, which was designated as a baseline transmission of 100%. Minimum transmission was determined by measuring the transmission of light through the stirred PRP, which was designated as a baseline transmission of 0%.

Platelet aggregation was induced by the addition of a 25- $\mu$ L aliquot of ADP or PAF to the PRP. Aggregation was recorded continuously as the increase in light transmission over time. Recording was continued until aggregation was complete, which was defined as the point when light transmission reached a plateau. When partial aggregation was followed by disaggregation, recording was stopped when light transmission reached 0%.

Two variables of platelet aggregation were evaluated (maximum aggregation achieved and initial aggregation velocity). Maximum aggregation achieved was measured as the total percentage increase in light transmission, and initial aggregation velocity was measured as the percentage increase in transmission per minute. Aggregation was performed in duplicate with high and low concentrations of ADP and PAF. When either aggregation variable differed markedly between the duplicate runs, the aggregation analysis was repeated in duplicate. Thus, there were 4 aggregation curves (in duplicate) for each dog.

**Fibrinogen concentration, 1-stage PT, and activated PTT**—An aliquot of PPP was evaluated to determine 1-stage PT, activated PTT, and concentration of fibrinogen (factor I). Testing was performed by personnel at the Animal Health Laboratory at the Atlantic Veterinary College in Guelph, ON, Canada, by use of standard methods. Fibrinogen concentration was quantified by use of thrombin time.<sup>25</sup>

**Quantification of clotting factors**—Activities of factors II, V, VII, VIII, IX, and X were quantified by use of plasma. A standard curve for activity was created by use of reference plasma obtained from clinically normal dogs and plasma deficient in each of the specific factors; curves were created by use of a manual photo-optical coagulation instrument.<sup>4</sup> The activities of factors II, V, VII, VIII, IX, and X were determined by combining plasma from each of the dogs in the study with plasma deficient in each of the specific factors and comparing the results with that of a standard curve. Activity of each of the factors was determined before (baseline) and after administration of each antimicrobial.

**Statistical analysis**—Baseline data obtained for each variable for each dog were combined to obtain a pooled pretreatment value for each dog, which was then subtracted from the posttreatment value. Changes between pretreatment and posttreatment values were analyzed by use of an ANOVA for a mixed model while controlling for period and carryover effects. When period and carryover did not have a significant effect, they were removed from the model used to test for differences among groups. When the overall *F* test was significant, then a post hoc Dunnett test was used to compare the posttreatment and pretreatment values.

For the 4 control dogs, baseline values were obtained for weeks 1, 3, 5, and 7, whereas posttreatment values were obtained for weeks 2, 4, 6, and 8. This was selected to coincide with the schedule for antimicrobial treatment in the 10 treated dogs. Changes for control dogs were analyzed by use of an ANOVA for a mixed model that accounted for repeated

measures over time. Data analysis included univariate analysis of the residuals. The Shapiro-Wilk test was used to confirm that residuals were normally distributed. For all tests, significance was set at a value of  $P \leq 0.05$ .

## Results

**Platelet count, Hct, and WBC count—**Antimicrobial administration did not result in a significant difference in platelet count before and after treatments. Baseline platelet counts for the first week were generally increased for all dogs; thus, they were not included in the analysis. In 4 dogs, platelet counts were slightly less than the laboratory reference range ( $117 \times 10^9$  cells/L to  $418 \times 10^9$  cells/L). These low platelet counts were detected once before antimicrobial administration and once each subsequent to cephalexin, doxycycline, and amoxicillin administration. No significant differences in Hct or WBC count were detected before and after antimicrobial administration.

**Bleeding time—**We did not detect a significant difference in platelet function before and after treatment administration as assessed by BMBT. Eighteen of 80 BMBT values were greater than the reference range ( $\leq 240$  seconds; Figure 1). Of these 18 values, 9 were recorded before and 9 were recorded subsequent to antimicrobial administration. Increased BMBT values after administration were recorded 3 times for doxycycline, 2 times for amoxicillin, 2 times for enrofloxacin, and 1 time for cephalexin. Evaluation of the increased BMBT values revealed a range of 242 to 456 seconds (mean, 312 seconds; median, 298 seconds).

**Platelet function as measured by use of a platelet-function analyzer and aggregometry—**We did not detect a significant difference in platelet function before and after antimicrobial administration as assessed via measurement of closure time by use of the platelet-function analyzer. We also did not detect significant differences in platelet function among any antimicrobials as assessed on the basis of maximal rate and degree of platelet aggregation in response to high and low doses of ADP and PAF.

**Values for 1-stage PT—**Cephalexin administration resulted in a significant ( $P = 0.046$ ) increase in 1-stage PT, compared with preadministration values. Although there was a pattern of a slight increase in 1-stage PT after administration of doxycycline, amoxicillin, and enrofloxacin, the increases were not significantly different from preadministration values (Figure 2). Eight measurements were slightly less than the laboratory reference range; all 8 were obtained before antimicrobial administration. No values obtained before or after antimicrobial administration were greater than the laboratory reference range.

**Values for activated PTT—**Administration of cephalexin and amoxicillin resulted in a significant ( $P = 0.031$  and  $0.046$ , respectively) increase in acti-

vated PTT, compared with preadministration values (Figure 3). A similar pattern was evident after administration of doxycycline, but the value after doxycycline administration was not significantly different from the preadministration value.

**Fibrinogen concentration—**Administration of enrofloxacin resulted in a significant ( $P = 0.008$ ) decrease in fibrinogen concentration, compared with preadministration values. Although there was a pattern of a slight decrease in fibrinogen concentration after administration of doxycycline, amoxicillin, and cephalexin, the decreased values did not differ significantly from the preadministration values (Figure 4). Eighteen values were greater than the laboratory reference range ( $\leq 2.3$  g/L). Thirteen were recorded before and 5 recorded subsequent to antimicrobial administration (1 time for doxycycline, 2 times for amoxicillin, and 2 times for cephalexin). Increased fibrinogen concentrations were recorded in 7 of 10 dogs and were detected throughout the study.

**Quantification of clotting factors—**We did not detect a significant difference in activities of factors II,

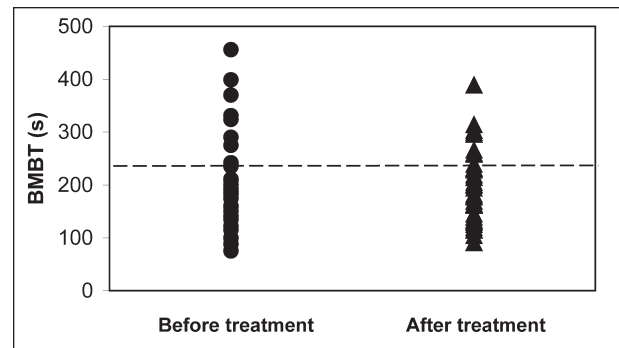


Figure 1—Values for BMBT before (circles) and after (triangles) oral administration of each of 4 antimicrobials to 10 healthy Beagles for 7 days. There was a minimum 7-day washout period between successive antimicrobials. Each symbol represents results for 1 dog. The upper boundary of the reference range for dogs (ie, 240 seconds) is indicated (horizontal dashed line).

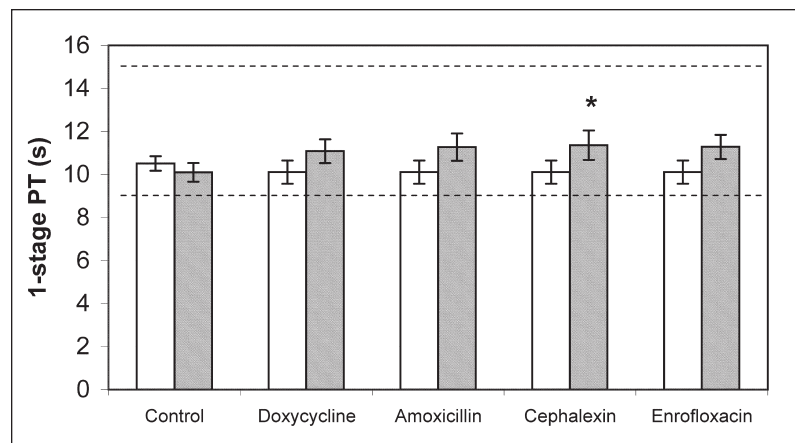


Figure 2—Mean  $\pm$  SD values for 1-stage PT before (white bars) and after (diagonal-striped bars) oral administration of each of 4 antimicrobials to 10 healthy Beagles for 7 days and for 4 untreated healthy control Beagles. The upper and lower boundaries of the reference range for dogs (ie, 9 to 15 seconds) are indicated (horizontal dashed lines). \*Within an antimicrobial, the value differs significantly ( $P \leq 0.05$ ), compared with values before antimicrobial administration.

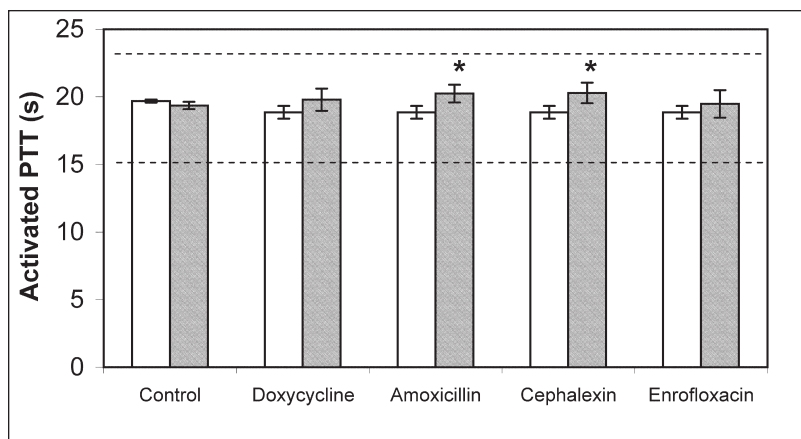


Figure 3—Mean  $\pm$  SD values for activated PTT before (white bars) and after (diagonal-striped bars) oral administration of each of 4 antimicrobials to 10 healthy Beagles for 7 days and for 4 untreated healthy control Beagles. The upper and lower boundaries of the reference range for dogs (ie, 15.0 to 23.5 seconds) are indicated (horizontal dashed lines). See Figure 2 for remainder of key.

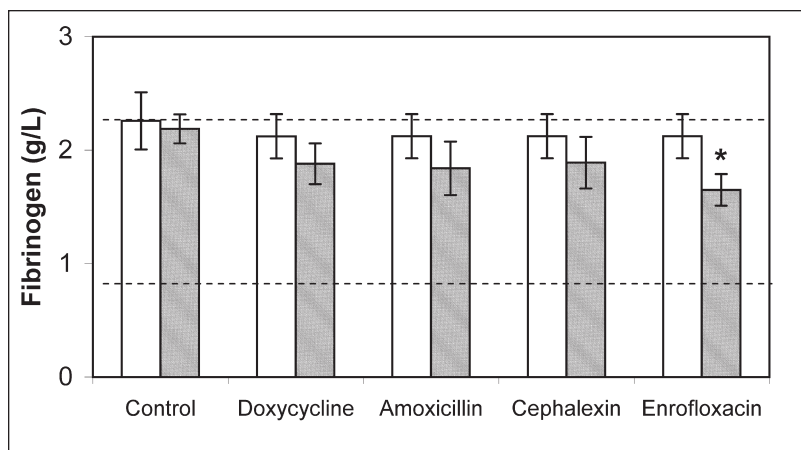


Figure 4—Mean  $\pm$  SD fibrinogen concentration before (white bars) and after (diagonal-striped bars) oral administration of each of 4 antimicrobials to 10 healthy Beagles for 7 days and for 4 untreated healthy control Beagles. The upper and lower boundaries of the reference range for dogs (ie, 0.9 to 2.3 g/L) are indicated (horizontal dashed lines). See Figure 2 for remainder of key.

V, VII, VIII, IX, or X before and after antimicrobial administration.

## Discussion

Reports of excessive hemorrhage after administration of penicillin compounds in humans led researchers to initiate studies<sup>7,24-26</sup> in an attempt to understand and quantify this interaction. Initial studies indicated that many drugs within the penicillin family, especially when administered at high dosages, resulted in inhibition of hemostasis in humans and dogs. In the interval since those studies, there has been much speculation and research into the pathophysiologic mechanisms of the inhibition, with the currently favored theory being that inhibition is attributable to a membrane modulation.<sup>25,26</sup>

Antimicrobials are commonly used in veterinary medicine, and their use is often indicated in patients with bleeding diatheses. An accurate understanding of their effects on hemostasis is important when selecting the class of antimicrobial to prescribe. It is difficult to

make these decisions on the basis of information contained in the literature because only a few studies<sup>6,27,28</sup> have been performed to evaluate the in vivo effects of antimicrobials at relevant dosages and durations in dogs. Two studies<sup>8,19</sup> were performed to determine the effect of IV administration of 2 doses of cephalosporins on hemostasis; the drugs were administered 1.5 to 3 hours apart to mimic a typical perioperative situation. In 1 study,<sup>25</sup> there was an irreversible inhibition of platelet aggregation that required exposure to the drug for at least 24 hours; therefore, it is possible that a longer duration of administration of cephalosporins may result in additional hemostatic abnormalities.

Evaluation of platelet function is challenging. Aggregometry, an in vitro assessment that uses whole blood or PRP, is laborious, not widely available, and therefore typically limited to controlled studies.<sup>25,29</sup> There currently is no standard for the doses of agonists used for aggregation studies.<sup>30</sup> Agonists selected for aggregometry in the study reported here were ADP and PAF, which can irreversibly induce aggregation in canine platelets.<sup>30</sup> The development of platelet-function analyzers has made it possible to investigate thrombocytopenia in clinically affected animals, and their use is likely to increase in the future.<sup>31,32</sup> The analyzer used in the study reported here has been validated for use in dogs and evaluated for detection of thrombocytopenia in dogs, including thrombocytopenia attributable to von Willebrand disease and the administration of aspirin.<sup>1,33,34,r</sup> The baseline closure times obtained before administration of antimicrobials in our study correlated well with the reported reference range.<sup>34</sup>

In the study reported here, analysis of platelet function by use of aggregometry and a platelet-function analyzer indicated that none of the antimicrobials administered resulted in thrombocytopenia. Therefore, administration of these antimicrobials to healthy animals should not be avoided solely on the basis of concerns about alterations in platelet function. All  $\beta$ -lactams do not affect aspects of hemostasis to the same degree<sup>9,24</sup>; thus, it is not possible to extrapolate results of the study reported here for other penicillins and cephalosporins.

Evaluation of platelet function by use of BMBT supported findings obtained by use of aggregometry and the platelet-function analyzer that indicated the antimicrobials evaluated did not cause alterations in primary hemostasis. However, the wide variability in BMBT in clinically normal untreated dogs and the large number of measurements in healthy dogs (18 of 80 measurements) that had a baseline BMBT greater than

the reference range should be mentioned. Although operator variability is 1 limitation of the BMBT, this was minimized in our study by use of a single operator trained in the technique, who was not aware of the treatment schedule. In addition, all dogs responded well to being positioned in lateral recumbency; therefore, restraint was not a problem.

The BMBT is considered a good screening test for platelet dysfunction in dogs once thrombocytopenia has been ruled out.<sup>1,21,35</sup> Authors of critical reviews<sup>21,31-33,36-39</sup> of bleeding time in humans, which included surveys of the literature and other supporting evidence of the use of bleeding time, concluded that bleeding time is an inaccurate test best used to interpret data for a population rather than for a specific person, with a sensitivity of only 50% to 65%. Direct comparisons of bleeding time in people have indicated improved sensitivity with the platelet-function analyzer<sup>40</sup> used in the study reported here. Analysis of data for our study revealed marked variability in BMBT in dogs, with clinically normal dogs having bleeding times that ranged from 1.25 to 7.60 minutes. However, other studies<sup>21,35,41-43</sup> in dogs have not detected this marked variability. The reason for the amount of variability in our study is not known; however, the small number of dogs may have been a factor (10 Beagles with 5 measurements of BMBT/dog). On the basis of a current report<sup>40</sup> for humans and results of our study, a prolonged BMBT should be considered in light of clinical signs and confirmed with aggregation analysis when possible. Additional studies are warranted to assess the accuracy of the BMBT.

Analysis of results of the study reported here indicated that platelet count was not affected by administration of antimicrobials. The initial baseline values were excluded from analysis because of a general increase in counts for all dogs. The cause of the increase in platelet count during initial baseline evaluations is not known; however, splenic contraction as a result of epinephrine release from excitement is a possible cause because that was the first week of data collection.

Initial studies<sup>27,44</sup> on the effects of antimicrobials on hemostasis focused primarily on thrombocytopathias typically caused by penicillins, and alterations in secondary hemostasis were not commonly seen. However, administration of cephalosporins causes a defect in secondary hemostasis via hypoprothrombinemia.<sup>16,17,45</sup> The cause of hypoprothrombinemia associated with the administration of cephalosporins is likely related to vitamin K; however, the relation to decreases in the amount of or total unavailability of vitamin K remains elusive.<sup>9,46</sup> Cephalosporins with an NMTT side chain or related methyl-thiadiazole side chain may be more prone to causing hypoprothrombinemia than cephalosporins that lack these side chains.<sup>8,16,46,47</sup>

Two studies<sup>8,19</sup> were conducted to investigate the effects of IV administration of 2 doses of cephalosporins, injected 1.5 to 3 hours apart, on hemostasis in clinically normal dogs. Investigators in both studies found that cephalothin resulted in inhibition of platelet aggregation as determined by use of

whole-blood aggregation analysis. Authors of one of those studies<sup>19</sup> concluded that cephalothin administration resulted in platelet dysfunction; however, measures of secondary hemostasis were not performed. In the other study,<sup>8</sup> investigators examined the effects of cephalothin, cefazolin, and cefmetazole on primary and secondary hemostasis. Analysis of results for that study indicated that primary hemostasis was altered after IV administration of cephalothin and cefmetazole but not after IV administration of cefazolin; however, there were discrepancies between results for whole-blood aggregation and BMBT. Furthermore, secondary hemostasis was assessed in that study after IV administration of 2 doses of cephalosporin and also after 1 week of SC administration of doses 3 times daily, and none of the variables for secondary hemostasis were altered for either administration protocol for cephalothin, cefmetazole, or cefazolin. Of the cephalosporins investigated, only cefmetazole contained an NMTT side chain. The reason results for these studies in dogs did not correspond to results in human studies and indicated an alteration in primary but not secondary hemostasis is unclear but may relate to species differences. Currently, there are too few studies to make definitive statements about the effect of cephalosporins on primary or secondary hemostasis in dogs.

Analysis of results for the study reported here revealed that administration of cephalixin, a cephalosporin without an NMTT side chain, caused a significant ( $P = 0.046$ ) increase in 1-stage PT, compared with preadministration values. Furthermore, this pattern of an increase in 1-stage PT was also evident after administration of doxycycline, amoxicillin, and enrofloxacin (Figure 2). In addition, cephalixin and amoxicillin administration resulted in a significant ( $P = 0.031$  and  $0.046$ , respectively) increase in activated PTT, compared with preadministration values. Again, a pattern of an increase in activated PTT was evident after administration of doxycycline and enrofloxacin (Figure 3). Evaluation of baseline values revealed that 8 measurements of 1-stage PT were less than the lower boundary of the reference range. The cause for these slightly low values is not known.

A significant ( $P = 0.008$ ) decrease in fibrinogen concentration was detected after enrofloxacin administration, compared with preadministration values. In addition, this pattern was evident for administration of doxycycline, amoxicillin, and cephalixin (Figure 4). Most of the fibrinogen concentrations obtained before antimicrobial administration were at the upper end of, or slightly greater than, the reference range for canines. This finding was not based on a time effect because preadministration samples were obtained throughout the course of the study. The increase in fibrinogen concentration was detected for most dogs in the study and was evident randomly throughout the duration of the study. Although inflammation could have resulted in an increase in fibrinogen concentration, this was unlikely because the dogs were closely monitored throughout the study and no abnormalities or evidence of infectious disease was detected. The dogs were not exposed to many infectious diseases because their

environment was routinely cleaned and monitored. Therefore, the cause for the mild increase in fibrinogen concentration in some clinically normal dogs is unknown. Antimicrobial administration did not result in alterations in any other variables evaluated, including prothrombin.

Differential diagnoses for an increase in 1-stage PT and activated PTT include a defect in the common pathway or deficiencies in multiple factors.<sup>2</sup> Deficiencies in multiple factors were not detected in the dogs of our study, and there was no evidence of a defect in the common pathway. The mild decrease in fibrinogen concentration may have been sufficient to cause a prolongation of 1-stage PT and activated PTT. Causes of hypofibrinogenemia include increased fibrinogen consumption (such as that resulting from disseminated intravascular coagulation), decreased synthesis of fibrinogen (such as that resulting from liver disease), and dysfibrinogenemia.<sup>2</sup> Antimicrobial administration to healthy dogs should not result in any of these disease states. Because fibrinogen concentrations are rarely measured in studies to evaluate the effects of antimicrobials on hemostasis, there is currently little data.<sup>27,44</sup> A study<sup>44</sup> in pregnant humans with underlying disease revealed an increase in fibrinogen concentration after antimicrobial administration; however, this was attributed to a false in vitro increase. In another study<sup>27</sup> in healthy dogs, investigators did not detect a change in fibrinogen concentration after penicillin administration.

Only once in the study reported here did the increases in 1-stage PT and activated PTT after antimicrobial administration result in a value greater than the reference range; therefore, these changes were not considered to be clinically relevant. Because this study was performed in healthy dogs, evaluation of the effect of antimicrobial administration on 1-stage PT, activated PTT, and fibrinogen concentration in dogs with underlying diseases that cause secondary hemostasis is warranted. In addition, evaluation of the effects of other antimicrobials on secondary hemostasis in dogs should be performed because there is currently a paucity of information in the veterinary literature.<sup>27,48</sup>

Dogs selected for use in the study reported here were healthy 10- to 18-month-old sexually intact Beagles; therefore, these results are most relevant to a population of young healthy Beagles. Additional studies are required to evaluate the effects of antimicrobials on a population of dogs with underlying disease. This is particularly warranted in animals with underlying disease processes, such as uremia and liver disease, that may alter hemostasis. For those diseases, changes in secondary hemostasis could result in substantial abnormalities that cause clinical bleeding.

Dosage and duration of administration for the 4 antimicrobials used in the study reported here were determined on the basis of dosing regimens typically used by general practitioners.<sup>20</sup> It is possible that additional or more marked hemostatic abnormalities would have been detected had the duration been extended substantially. Because the dosage selected was at the high end of those currently used for treatment, evaluation of these antimicrobials at higher dosages does not appear to be warranted.<sup>20</sup>

Oral administration of therapeutic amounts of doxycycline, amoxicillin, cephalexin, and enrofloxacin to healthy dogs for a period of 7 days did not result in platelet dysfunction or a change in platelet count but did cause slight alterations in measures of secondary hemostasis. No alterations in specific factors were evident. Some of these changes were statistically significant; however, none appeared to be clinically relevant in healthy dogs. Additional studies are warranted to assess the effects of these antimicrobials on dogs with hemostatic abnormalities. Studies are also warranted to evaluate the effects of other antimicrobials on hemostasis in healthy dogs and dogs with underlying diseases.

- a. PFA-100, Dade Behring Canada Inc, Mississauga, ON, Canada.
- b. Apo-doxy, Apotex Inc, Toronto, ON, Canada.
- c. Apo-amoxi, Apotex Inc, Toronto, ON, Canada.
- d. Novo-lexin, Novopharm, Toronto, ON, Canada.
- e. Baytril, Bayer Inc, Toronto, ON, Canada.
- f. Advia 120, Bayer Inc, Toronto, ON, Canada.
- g. Simplate, bioMérieux Canada Inc, St Laurent, QC, Canada.
- h. Filter paper, Whatman Inc, Clifton, NJ.
- i. Transfer pipette, Fisher Scientific, Pittsburgh, Pa.
- j. Research pipette, Eppendorf, Mississauga, ON, Canada.
- k. Centrifuge tubes, Fisher Scientific, Pittsburgh, Pa.
- l. Unopette microcollection system, Becton-Dickinson, Franklin Lakes, NJ.
- m. Bright-Line hemacytometer, American Optical, Buffalo, NY.
- n. Equine muscle adenosine 5'-diphosphate, Sigma-Aldrich Co, St Louis, Mo.
- o. Synthetic PAF-16, EMD Biosciences, San Diego, Calif.
- p. Chrono-log 440VS, Chrono-log Corp, Haverton, Pa.
- q. Coag-a-mate XM, bioMérieux Canada Inc, St Laurent, QC, Canada.
- r. Ferrand G, Girod A, Bonnefond-Rebeix C, et al. PFA-100 evaluation in healthy dogs and in a population of dogs with high prevalence of von Willebrand's disease (abstr). *Vet Clin Pathol* 2002;31:198.

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