# USE OF VISCOELASTIC COAGULATION TESTING IN MEGACHIROPTERA REVEALS HIGH VARIABILITY IN CLOT KINETICS

College of
Veterinary Medicine
UNIVERSITY of FLORIDA
Emily E. Brenner, VMD,<sup>1\*</sup> Amy B. Alexander, DVM, Dipl ACZM,<sup>1</sup> Leonel A. Londoño DVM, Dipl ACVECC,<sup>2</sup> Nicole I.
Stacy, DVM, Dipl ACVP,<sup>1</sup> Jorge A. Hernandez, DVM, PhD,<sup>3</sup> Sarah E. Crevasse,<sup>1</sup> and James F. X. Wellehan Jr., DVM, MS,





Abstract

Megachiroptera is a mammalian suborder that includes old world fruit bats and flying foxes. Common diseases among captive megachiroptera include liver disease, (e.g., iron storage disease), kidney disease (e.g., protein-losing nephropathy), and heart disease (e.g., dilated cardiomyopathy).<sup>4-6,8</sup> These conditions can be significantly affected by or directly affect coagulation.<sup>7,11,12</sup> A large number of geriatric megachiroptera in captivity have osteoarthritis that is treated with longterm nonsteroidal anti-inflammatory drugs (NSAIDs). Studies in humans and dogs have shown that NSAIDs negatively affect clot formation and strength.<sup>2,3,9</sup> Hemostatic variables were evaluated in captive large flying foxes (*Pteropus vampyrus*) (n=20) and variable flying foxes (*Pteropus* hypomelanus) (n=10) using point-of-care viscoelastography,<sup>a</sup> as well as platelet counts, prothrombin time [PT], and partial thromboplastin time [PTT].<sup>1,10</sup> The results showed marked variability among both clinically normal and NSAID treated populations. Viscoelastic variables, platelet count, and clotting times from the control group were compared to an age and sex-matched population of bats treated with NSAIDs for underlying osteoarthritis or allergic dermatitis. No significant differences were noted in hemostatic variables between treated and untreated groups. The results from this study indicate that clot kinetics vary widely amongst megachiropterans and it is recommended that clinicians establish subject-based reference intervals for individual bats to detect early changes in hemostasis during injury or disease.



## Results

- 1. Large variability in all viscoelastic parameter values noted in healthy bats.
- No correlation between viscoelastic parameter values and clotting times or platelet counts
- 3. CT and PTT values were higher ( $P \le 0.05$ ) in *P. vampyrus*,

<sup>a</sup> Entegrion Inc, Durham, NC 27703, USA 32607, USA

Objectives

Figure 1: An adult *Pteropus vampyrus* or Malayan Flying fox



compared to *P. hypomelanus*, and PT values were higher (p = 0.02) in *P. hypomelanus*, compared to *P. vampyrus*. Viscoelastic values were not different ( $p \ge 0.01$ ) between males and females or between age age-class groups ( $\ge 0.06$ ).

4. Comparisons of viscoelastic parameters between healthy bats with NSAID-medicated bats were not statistically significant.



Figure 4 Correlation between CFT and PTT (R = 0.04)



- 1. Establish reference intervals for viscoelastic parameter values in healthy bats
- 2. Examine the correlation between viscoelastic parameter values and clotting times or platelet counts
- 3. Compare viscoelastic parameter values amongst species, age class and sex
- 4. Compare viscoelastic parameter values between healthy untreated bats and bats treated with NSAIDs

 $\frac{CFT}{436} \frac{17.441}{116-301} + \frac{1}{116-301} + \frac{1}{116-30$ 

Figure 2: Examples of VCM tracings of bats showing wide variety in clotting kinetics

# Parameters

#### List of VCM parameters and description

Parameter	Unit of measurement	Parameter description
CT(Clot Time)	Seconds (s)/ Minutes (m)	The clot time is the time from the beginning of the test until the time when an amplitude of 1% above the baseline is achieved.
CFT (Clot Formation Time)	Seconds (s)/ Minutes (m)	The clot formation time is the time between 1% amplitude and 10% amplitude of the clotting signal.
Alpha (Alpha-Angle)	Degrees (*)	The alpha angle is defined as the angle between the time axis and the tangent to the clotting curve through the 1% amplitude point. It describes the kinetic of clotting.
MCF (Maximum Clot Formation)	VCM Units	The Maximum Clot Formation is the measure of the firmness of the clot and therefore the clot quality. It is the maximum amplitude that is reached before the clot is dissolved by fibrinolysis

Figure 5: Correlation between PPT and CT (R = 0.37)

# Conclusions

Species specific reference ranges should be used when evaluating coagulation in megachiroptera due to significant differences in viscoelastic values (CT) and clotting times (PT, PTT) between megachiropteran species.

Due to the high individual variability seen in viscoelastic values amongst bats the authors recommend that individual reference ranges for viscoelastography should be established to detect early changes in coagulation.

There is poor correlation between clotting tests, therefore, when evaluating hemostasis in bats the authors recommend including viscoelastography, clotting times and platelet count as part of the diagnostic work up.

# Materials and Methods

Animals: 30 individuals, 2 species, aged 6-32, both sexes included

Clotting tests evaluated: Viscoelastography (Entegrion VCM Vet), clotting times (PT, PTT), platelet count

Blood collected from 15 healthy bats and 15 bats treated with antiinflammatories (meloxicam or aspirin) for clotting tests

Objective 1 + 4: Viscoelastic parameter values were calculated and reported as mean ± standard deviation and median (first, third quartiles). Data for the variables of AA and PTT were not normally distributed.

Objective 2: The correlation between viscoelastic parameter values and clotting times or platelet counts was examined by constructing scatter diagrams and using simple regression analysis.

Objective 3: Among non-treated bats, the distributions for viscoelastic parameter values were compared by species (Hypo, Vamp), sex (male, female), and age-class (6-16 years-old, 20-32 years-old) by using the Student T Test or the non-parametric Wilcoxon Rank Sum Test. Values of p < 0.05 were considered statistically significant.

A10 & A20	VCM Units	The A10 and A20 represent the clot firmness.
(Amplitude at		The A10 and A20 are the amplitude at 10
10 and 20 mins)		minutes and 20 minutes after clot time.
LI30 & LI45	%	The LI30 and LI45 are the amplitude of the clot
(Lysis index at		at 30 and 45 minutes after clot time as a
30 and 45 mins)		percentage of the MCF.

and the clot firmness falls agai

#### The VCM trace



Figure 3: List of VCM parameters and descriptions

### References

1. Benes J, Zatloukal J, Kletecka J. Viscoelastic methods of blood clotting assessment – a multidisciplinary review. Front Med. 2015;2:62.

2. Brainard BM, Meredith CP, Callan MB, Budsberg SC, Shofer FS, Driessen B, Otto CM. Changes in platelet function, hemostasis, and prostaglandin expression after treatment with nonsteroidal anti-inflammatory drugs with various cyclooxygenase selectivities in dogs. Am J Vet Res. 2007;68(3):251-257.

Dannhardt G, Kiefer W. Cyclooxygenase inhibitors – current status and future prospects. Eur J Med Chem. 2001;36:109-126.
Deem SL, Heard DJ, Clippinger TL, Buergelt CD. Cranial edema associated with a protein-losing nephropathy in a golden-mantled flying fox (*Pteropus pumilus*). J Zoo Wildl Med. 1999;30:126-131.

5. Farina LL, Lankton JS. Chrioptera. In: Terio KA, McAloose D, St. Leger J (eds.) Pathology of wildlife and zoo animals. San Diego (CA):Academic Press; 2018. P. 607-632

6. Heard DJ, Buergelt CD, Snyder PS, Voges AK, Dierenfeld ES. Dilated cardiomyopathy associated with hypovitaminosis E in a captive collection of flying foxes (Pteropus spp.). J Zoo Wildl Med. 1996;27(2):149-157.

7. Janko N, Majeed A, Kemp W, Roberts SK. Viscoelastic tests as point-of-care tests in the assessment and management of bleeding and thrombosis in liver disease. Semin Thromb Hemost. 2020;46(06):704-715.

8. Leone AM, Crawshaw GJ, Garner MM, Frasca S, Stasiak I, Rose K, Neal D, Farina LL. A retrospective study of the lesions associated with iron storage disease in captive Egyptian fruit bats (*Rousettus aegyptiacus*). J Zoo Wildl Med. 2016;47:45-55.

9. Martini AK, Rodriguez CM, Cap AP, Martini WZ, Dubick MA. Acetaminophen and meloxicam inhibit platelet aggregation and coagulation in blood samples from humans. Blood Coagul Fibrinolysis. 2014;25(8):831-837.

10. Shen L, Tabaie S, Ivascu N. Viscoelastic testing inside and beyond the operating room. J Thorac Dis. 2017;9(Suppl 4):S299-S308. 11. Tarnow I, Falk T, Tidholm A, Martinussen T, Jensen AL, Olsen LH, Pedersen HD, Kristensen AT. Hemostatic biomarkers in dogs with chronic congestive heart failure. J Vet Intern Med. 2007;21:451-457.

12. White CR, Langston C, Hohenhaus AE, Lamb K, Hackner S, Fox PR. Evaluation of the relationship between clinical variables and thromboelastographic findings in dogs with protein-losing nephropathy. J Vet Emerg Crit Care. 2016;26(1)74-79.

Acknowledgements: The authors would like to thank the Lubee Bat Conservancy and Entegrion VCMVet for their support.