

ORIGINAL RESEARCH

Plasmatic coagulation and fibrinolysis in healthy and *Otostrongylus*-affected Northern elephant seals (*Mirounga angustirostris*)

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Key Words

Coagulopathy, disseminated intravascular coagulation, thromboelastography

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Background: Prepatent *Otostrongylus* arteritis results in hemorrhagic diathesis in free-ranging Northern elephant seals (*Mirounga angustirostris*) attributed to aberrant larval migration of the lungworm, *Otostrongylus circumlitus*. Clinical signs are often nonspecific, including lethargy, anorexia, and blepharospasm, but can progress to spontaneous frank hemorrhage and death within 72 hours of onset. Previously published case reports describe coagulopathy with prolonged PT and APTT, normal to elevated platelet counts, normal antithrombin concentrations, and low concentrations of fibrinogen degradation products. Disseminated intravascular coagulation was proposed as the cause of hemorrhage, but is inconsistent with some of the reported clinicopathologic changes.

Objective: The purpose of this study was to compare plasmatic coagulation and fibrinolysis in healthy and *Otostrongylus*-affected elephant seals, in order to identify potential therapy. We hypothesized that hyperfibrinolysis contributed to hemorrhage in these cases.

Methods: Citrated plasma samples were collected from 3- to 4-month-old Northern elephant seals in a wildlife rehabilitation hospital. The sampled population included 25 healthy, prerelease seals and 32 clinically ill seals diagnosed with presumptive *Otostrongylus* arteritis. Twenty-one of the included seals had *Otostrongylus* infestation confirmed at necropsy. Standard coagulation tests and plasma thromboelastography were performed for a complete assessment of coagulation and fibrinolysis.

Results: Northern elephant seals with definitive *Otostrongylus* infestation were hypocoagulable and hypofibrinolytic compared to healthy controls.

Conclusions: Results were most consistent with disseminated intravascular coagulation. Treatment with antifibrinolytic drugs to control hemorrhage may be unrewarding; alternative therapies such as plasma transfusions or coagulation factor concentrates should be investigated.

Introduction

Prepatent *Otostrongylus* arteritis results in a hemorrhagic diathesis of weanling free-ranging Northern elephant seals (*Mirounga angustirostris*; NES) attributed to aberrant larval migration of the lungworm *Otostrongylus circumlitus*. First described in 1996¹, the disorder has been diagnosed in 12% of juvenile NES admitted to The Marine Mammal Center (TMMC) in Sausalito, California from 2000 to 2013. Case fatality at TMMC approaches 90%, comprising 26% of annual NES mortalities at TMMC (S.J., unpublished

data). The syndrome may present clinically as lethargy, anorexia, dehydration, blepharospasm, congested mucous membranes, and clinical hemorrhage from nares, mouth, or injection sites. There is no definitive antemortem test for prepatent *Otostrongylus* arteritis, as the syndrome develops during the initial larval migration stage, prior to parasite maturation, reproduction, and release of first-stage larvae. Detection of *Otostrongylus* larvae in fecal examinations is only reported in older elephant seals (5–6 months) without the prepatent arteritis syndrome, suggesting that some seals survive the initial infection.² On gross

necropsy, affected NES have *O circumlitus* larvae and adults in the heart and pulmonary vasculature, accompanied by multifocal hemorrhage and pulmonary thrombosis.² Histologically, there are a severe suppurative arteritis, disseminated microthrombi, and evidence of septicemia.² Hemorrhage was attributed to disseminated intravascular coagulation (DIC), as a complication of arteritis induced by larval parasite migration through the vasculature.²

In DIC, activation of coagulation pathways results in widespread thrombosis leading to depletion of coagulation factors, uncontrolled hemorrhage, and organ failure.³ Conditions that can result in DIC include trauma, neoplasia, sepsis, and vascular disease, among others.⁴ Diagnosis of DIC relies on assessment of patient history, underlying disease, and multiple clinicopathologic variables. The International Society of Thrombosis and Hemostasis (ISTH) scoring algorithm for diagnosis of overt DIC includes platelet count, PT, fibrinogen concentration, and fibrinogen degradation products (FDPs), with thrombocytopenia and elevated FDPs being the most commonly reported abnormalities.^{4,5} Additional findings typical for a DIC diagnosis include prolonged APTT, and reduced activity of antithrombin and protein C.⁴ In reported cases of *Otostrongylus* arteritis, certain laboratory variables were inconsistent with a diagnosis of DIC, including normal or elevated platelet counts, low levels of fibrinogen degradation products (FDPs), and normal antithrombin activity.¹ These conflicting results obscure the clear pathogenesis of this syndrome.

There are currently no specific treatments for *Otostrongylus* arteritis that have proven safe or effective. Antiparasitic medications, including ivermectin and fenbendazole, have been applied in the past, but in the authors' experience contributed to peracute death, possibly due to worm fragmentation and embolization, as reported in canine and feline heartworm disease.^{6,7} Northern elephant seals suspected of *Otostrongylus* arteritis are currently not treated with antiparasitic medications, but rather supporting therapy directed at the clinical syndrome due to prepatent infection. This supportive care includes subcutaneous fluids, gavage feeding, and antibiotics against secondary bacterial infections. Sick NES progressing to frank hemorrhage in the acute period of 1–3 days have only limited treatment options and a poor prognosis. Plasma transfusions, the treatment of choice for DIC, are challenging in a large-scale wildlife rehabilitation setting due to cost, personnel required, and lack of suitable healthy donors. The authors have investigated aminocaproic acid, a lysine analog antifibrinolytic drug for

control of hemorrhage in *Otostrongylus* cases, but this treatment has not yet been subjected to a clinical trial.⁸ An explicit description of the mechanism leading to hemorrhage in *Otostrongylus* seals could allow for the recommendation of appropriate, specific medical therapy.

Hyperfibrinolysis has been identified as a cause of hemorrhage in parasitic diseases, such as those due to *Schistosoma*, *Dirofilaria*, and *Ancylostoma*.^{9–11} We hypothesized that a hyperfibrinolytic state induced by *Otostrongylus* infestation contributed to the hemorrhagic diathesis in these animals. Specifically, we hypothesized that citrated plasma from *Otostrongylus*-affected seals would exhibit a hypocoagulable and hyperfibrinolytic profile when compared to healthy control plasma.

To investigate this hypothesis, we used plasma thromboelastography (TEG) to evaluate coagulation and fibrinolysis in *Otostrongylus*-infested NES, compared to a healthy reference population. Thromboelastography is an in vitro test of clotting and fibrinolysis, run with citrated whole blood or citrated plasma. The sample is placed in a cup containing calcium chloride for clotting activation. A pin is suspended in the sample, and the cup is rotated. Clot formation results in fibrin strands connecting the pin to the sides of the cup, generating torsion on the pin. Increased clot strength produces increased torsion, and subsequent clot lysis decreases torsion. The change in torsion on the pin is reported by TEG software, generating a TEG curve that outlines the formation and breakdown of the clot over time (Figure 1).¹² The data are quantified by both measured and calculated curve variables (Table 1). In both research and clinical applications, clot activators such as tissue factor or kaolin are often included to expedite clot formation, which tends to produce less variability than nonactivated assays.¹³ As the process of fibrinolysis can be too slow for practical analysis, several authors have also modified the TEG assay with tissue plasminogen activator (tPA) to expedite lysis and facilitate measurement of lysis variables.^{14–16}

In veterinary medicine, TEG has been used for both clinical and research applications in dogs^{17–20}, horses²¹, Asian elephants²², manatees²³, Elephant seals⁸, and others.¹² Guidelines for use in domestic species were published in 2014²⁴, but universal assay methods are not established for exotic species. For this study, we utilized an activated and tPA-modified plasma TEG to analyze coagulation and fibrinolysis in *Otostrongylus*-affected Elephant seals in comparison to a healthy reference population.

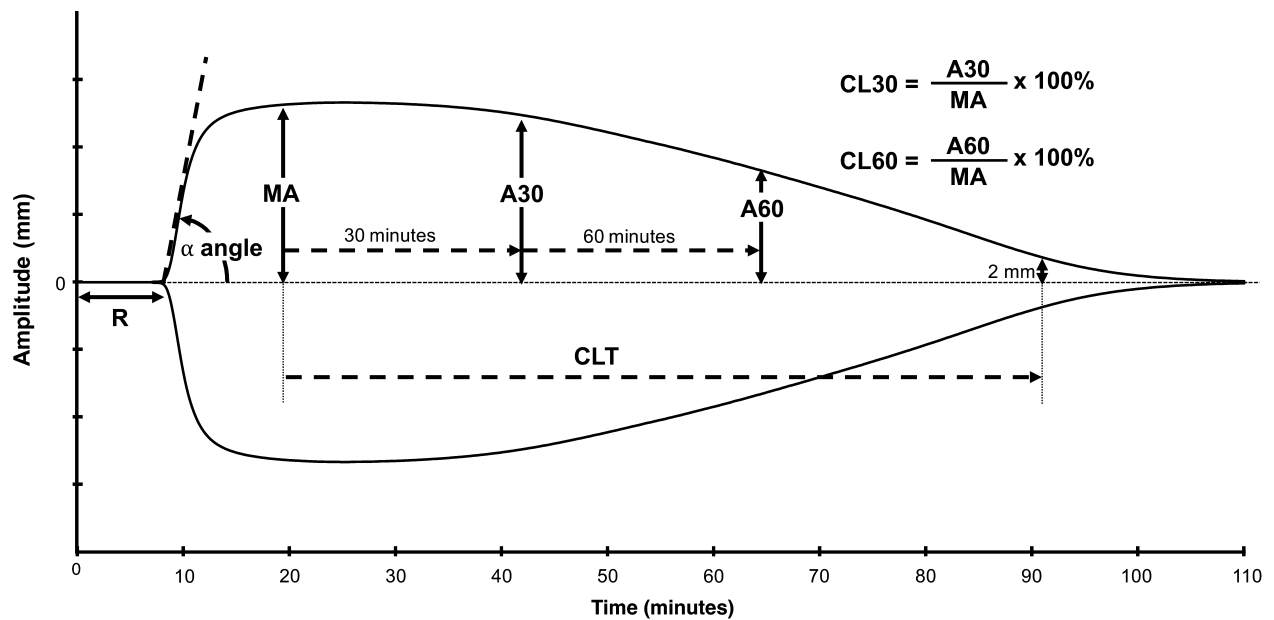


Figure 1. The thromboelastograph curve reveals change in clot strength over time as change in amplitude. Curve amplitude increases as coagulation occurs, and decreases during fibrinolysis. Variables are defined in Table 1.

Table 1. Definition of thromboelastograph variables.

Parameter	Definition
R (seconds)	Clot initiation; measured from start of assay to time that amplitude = 2 mm
α (deg)	Rate of clot formation; angle of the line tangent to the curve between 2 and 20 mm
MA (mm)	Maximum amplitude, indicative of maximum clot strength
A30, A60 (mm)	Amplitude 30 and 60 minutes after MA is reached
CL30, CL60 (%)	Clot strength at 30 and 60 minutes post-MA, calculated as percent amplitude remaining
LY30, LY60 (%)	Lysis at 30 and 60 minutes post-MA; calculated as percent change in area of curve
CLT (min)	Clot lysis time; time from MA to amplitude = 2 mm
<i>nat</i>	Subscript indicates native TEG with no modifiers added
<i>Tpa</i>	Subscript indicates tPA-modified TEG

Materials and Methods

Study population

The reference population consisted of 25 clinically healthy, prerelease, 3- to 4-month-old NES that were rehabilitated at TMMC in May and June 2014. All seals were determined to be healthy based on physical examination, biochemical profile, and a CBC. All study seals were released in the summer of 2014.

The samples from the study population were prospectively drawn from 3- to 4-month-old NES presenting to TMMC in 2015. Any NES presumptively diagnosed with *Otostromylus* arteritis using

standard clinical criteria established by TMMC was sampled for the study. As disease develops during the prepatent period, before the parasite can be detected in fecal examinations, diagnosis and institution of therapy are based on interpretation of a set of physical examination and clinicopathologic changes. Criteria used by the TMMC veterinary staff included one or more of the following: frank hemorrhage from an orifice, elevated WBC count ($>40,000/\mu\text{L}$) with band neutrophilia ($>5\%$ band neutrophils), elevated activity of GGT or AST, or acute onset of non-specific signs such as lethargy, anorexia, congested conjunctiva, or regurgitation. Variables reported for each study seal included initial clinical signs, presence or absence of external frank hemorrhage, final diagnosis, final outcome (survival, death, and euthanasia), and biochemical and CBC variables. Retrospectively, cases were reviewed and any animal that ultimately received a diagnosis other than *Otostromylus* was excluded from further analysis. Any seals that were euthanized or died during treatment underwent a gross postmortem examination, producing a subset of the study population with definitive *Otostromylus* infestation.

Sample collection

The control population was sampled opportunistically at time of routine prerelease laboratory examination. The study population was sampled at time of diagnosis

with presumptive *Otostrongylus* arteritis, prior to institution of therapy. The sampling protocol was approved by the Institutional Animal Care and Use Committee at both TMMC and Cornell University.

For all seals, blood was collected from the epidural sinus using a 20 Ga 1.5" needle on a Vacutainer set (Becton Dickinson, Franklin Lakes, NJ, USA). Samples for coagulation testing were collected directly into 2 4 mL citrated tubes and placed on ice until processing. Citrated plasma was obtained by centrifugation of whole blood at 2500g for 20 min, and was stored in 1.5 mL cryovial aliquots at -80°C . Frozen plasma was stored at TMMC and shipped on dry ice to Cornell for analysis.

Routine coagulation testing

Citrated plasma (0.5 mL) from each seal was submitted to the Comparative Coagulation Laboratory at the Cornell University Animal Health Diagnostic Center for a routine coagulation panel, including PT, APTT, antithrombin III (ATIII) activity, fibrinogen concentration, and D-dimer. All assays were performed using an automated hemostasis instrument (STA Compact; Diagnostica Stago, Parsippany, NJ, USA) and commercial reagents (aPTT reagent, Dade Actin FS; Dade Behring, Newark, DE, USA; PT reagent, Thromboplastin LI; Helena Diagnostics, Beaumont, TX, USA; fibrinogen reagent, Fibrinogen; Diagnostica Stago). Both PT and APTT had upper limits of quantification of 90 s and 180 s, respectively. For the purpose of quantitative analysis, results beyond the limit of PT quantification were assigned a value of 90 s, and results beyond the limit of APTT quantification were assigned a value of 180 s. Antithrombin activity was measured based on inhibition of thrombin (anti-IIIa assay) using a chromogenic kit (Stachrom ATIII; Diagnostica Stago) and D-dimer concentration in ng/mL was measured in a quantitative, immunoturbidometric assay configured with monoclonal anti-human D-dimer antibodies (HemosIL; Instrumentation Laboratories, Bedford, MA, USA) according to the manufacturer's instructions. The standard curves for determination of fibrinogen and AT were derived from a calibrated human plasma standard (STA Unicalibrator; Diagnostica Stago). The D-dimer assay was performed using the manufacturer's human D-dimer standard (HemosIL, D-dimer calibrator; Instrumentation Laboratories).

Thromboelastography

Each TEG assay was performed using the Thrombelastograph Analyzer 5000 (Haemonetics Corporation,

Braintree, MA, USA), with standard disposable cups and pins. All assays were activated with RapidTEG (Haemonetics Corporation), a commercial activator containing tissue factor and kaolin. This activator was selected after an in-house analysis found insufficient activation with use of either tissue factor or kaolin as lone activators in NES plasma (data not presented). Two assays were run for each seal: an activated native assay with no modifiers added, and an activated assay modified with 1000 U/mL (tPA). All assays were run for 60 min after maximum amplitude (MA) was reached. The TEG output variables are defined in Table 1.

For the reference population, all assays were run in duplicate, in 2 TEG cups run simultaneously, and final TEG variables were calculated as an average of the duplicates. For these samples, 700 μL aliquots of frozen plasma were thawed to 37°C in a warm water bath, and used within 15 min of thawing. RapidTEG reagent was reconstituted following the manufacturer's directions, and 20 μL were added to the 700 μL plasma aliquot. For the tPA-modified assay, 20 μL of diluted tPA were added to the aliquot to produce a final concentration in each TEG cup of 1000 U/mL tPA. For the native assays, 20 μL HEPES + 2% bovine albumin were used in place of tPA as an inactive buffer solution. From this mixture, 340 μL were pipetted into each of 2 TEG channels containing 20 μL of 10% calcium chloride (360 μL total volume in the TEG cup) as per the manufacturer's directions.

For the study population, no duplicates were run due to limited plasma volume available, and each assay was run in a single TEG cup. Plasma was thawed as previously described, and divided into 2 350 μL aliquots, one aliquot for the native assay and one for the tPA-modified assay. For the tPA-modified assay, 10 μL tPA and 10 μL RapidTEG were added to a 350 μL plasma aliquot to produce a final concentration in the TEG cup of 1000 U/mL tPA. For the native assay, 10 μL HEPES + 2% bovine albumin were added in place of tPA. From each mixture, 340 μL were pipetted into one of 2 TEG channels containing 20 μL of 10% calcium chloride (360 μL total volume in the TEG cup).

Statistical analysis

Statistical analysis included only NES with a definitive diagnosis of *Otostrongylus* infestation based on gross necropsy. Study seals with only a presumptive clinical diagnosis, but no necropsy confirmation, were excluded from analysis to prevent confounding by misclassification. In addition, definitive cases were further

classified as presenting with or without clinical hemorrhage. All data were tested for outliers using the Dixon statistic,²⁵ and duplicated TEG assays were tested for significant differences using a Student's *t*-test. Coagulation assay and TEG variables were compared between definitive cases and controls using the Wilcoxon rank-sum test, with a significance level of $P < .05$. Analysis was performed in JMP (SAS Institute Inc., Cary, NC, USA).

Results

Study population

Forty-three NES met the initial clinical criteria for *Otostrongylus* arteritis, and were prospectively sampled for the study. Of these, 11 were retrospectively excluded based on limited clinical criteria for inclusion, or definitive diagnosis of a disease other than *Otostrongylus*. The excluded population included 6 NES that survived to release without sufficient clinical criteria for *Otostrongylus* arteritis diagnosis, and 5 NES that died or were euthanized, and were diagnosed with a disease other than *Otostrongylus* infection. No excluded NES had *Otostrongylus* worms at necropsy. Of the 32 remaining NES considered clinical cases, 10 survived to release (31%), 15 died (47%), and 7 were euthanized (22%). Of the 22 clinical cases undergoing necropsy, 21 (95%) were confirmed to be infested with *Otostrongylus*. Only these 21 cases with definitive diagnosis were used in the final statistical analysis.

A total of 17 (52%) presumptively diagnosed cases exhibited frank hemorrhage. Of seals with clinical hemorrhage, 4 survived to release (24%) and 13 died or were euthanized (76%); 12 of 13 seals with hemorrhage (92%) had *Otostrongylus* on necropsy. There was no significant difference in survival in seals with or without clinical hemorrhage (chi-squared test, $P = .53$), and of cases with necropsy, those with a history of clinical hemorrhage were not more likely to have *Otostrongylus* infestation than cases without hemorrhage (chi-squared test, $P = .88$).

Routine coagulation testing

Standard coagulation test results for the control ($n = 25$), the definitive case ($n = 21$), and the definitive case subset with clinical hemorrhage ($n = 12$) are provided in Table 2. No outliers were detected. There were significant coagulation differences between groups. Affected seals appeared hypocoagulable, with prolonged PT and APTT. D-dimers were

significantly increased and fibrinogen concentration was significantly decreased, consistent with consumptive coagulopathy.

Thromboelastography

Thromboelastograph results are provided in Table 3. Duplicated assays from control seals showed good agreement with no significant intra-assay variation; values were averaged for analysis. Affected case seals were hypocoagulable, with prolonged R time, lower α , and lower MA compared to control seals (Table 1). As expected, there was minimal detectable fibrinolysis in either group using native TEG, but significant differences in fibrinolysis were apparent with the tPA-modified TEG. By this technique, affected seals appeared hypofibrinolytic, with lower LY30 and LY60 and concurrently higher CL30 and CL60. The subset of case seals with clinical hemorrhage tended to exhibit higher lysis variables than cases without hemorrhage, but the difference was not statistically significant (Table 3).

Discussion

This study expands on previous reports of coagulopathy in NES affected with prepatent *Otostrongylus*

Table 2. Medians (range) of standard hematology and coagulation variables in healthy control and *Otostrongylus*-affected Northern elephant seals. Results for all cases and for a subset of cases presenting with frank hemorrhage are reported.

Variable	Control ($n = 25$) Median (range)	All definitive cases ($n = 21$) Median (range)	Cases with frank hemorrhage ($n = 12$) Median (range)
Hematocrit (%)	55 (46.1–63.7)	53.1 (22.8–58.5)	53 (22.8–58.3)
Platelet count (thousands/ μ L)	373 (268–619)	178 (12–533)	201 (60–533)
PT (seconds)	15.6 (13.2–27.1)	28.3 (13.1–90.0)*	28.6 (13.7–90)*
APTT (seconds)	23.8 (16.3–41.7)	39 (25.5–180)*	40.3 (25.5–180)*
Antithrombin III (%)	85 (73–98)	85 (57–114)	83 (57–102)
Fibrinogen (mg/dL)	148 (72–211)	72 (0–193)*	96 (35–191)*
D-dimer (ng/mL)	0 (0–177)	375 (0–1386)*	485 (95–1386)*

*Indicates case variables significantly different from controls by the Wilcoxon sum rank test, at $P = .001$.

Table 3. Medians (range) of thromboelastography (TEG) variables in healthy control and *Otostrongylus*-affected Northern elephant seals.

Variable	Control (n = 25) Median (range)	Definitive cases (n = 21) Median (range)	Cases with frank hemorrhage (n = 12) Median (range)
R _{nat} (seconds)	0.65 (0.4–7.4)	1.1 (0.5–148.5)*	1.2 (0.6–148.5)*
α _{nat} (degree)	73.8 (35.8–81.2)	60.4 (0.2–79.3)	64.5 (23.7–79.3)
MA _{nat} (mm)	16.6 (6.5–30.9)	9.9 (2–23.5)*	10.8 (3.2–23.4)
CL30 _{nat} (%)	100 (50–100)	100 (100–100)	100 (100–100)
LY30 _{nat} (%)	0 (0–3.55)	0 (0–0.1)	0 (0–0)
CL60 _{nat} (%)	100 (0–100)	100 (100–100)	100 (100–100)
LY60 _{nat} (%)	0 (0–2.5)	0 (0–0.1)	0 (0–0)
CLT _{nat} (min)	71.6 (4.75–174.9)	174.9 (0–178.1)*	—
CL30 _{tpa} (%)	10.3 (0.2–90.7)	45.5 (2.6–100)*	32.7 (2.6–100)
LY30 _{tpa} (%)	33.0 (1.9–62.3)	16 (0–51.4)*	21.8 (0–49.5)
CL60 _{tpa} (%)	1.0 (0.55–40.3)	3.5 (0.5–100)*	2.25 (0.8–100)
LY60 _{tpa} (%)	67.9 (20.0–81.1)	51.7 (0–76.4)*	56.9 (0–75.5)
CLT _{tpa} (min)	28.6 (18.8–64.5)	38 (95.8–136.3)	—

*Indicates case variables significantly different from controls by the Wilcoxon sum rank test, at $P < .05$.

arteritis, including the initial case report and one retrospective case series of 5 NES.^{1,2} These previous cases documented prolonged PT and APTT, often with normal platelet counts, and pathologic findings of frank visceral and cavitory hemorrhage and suppurative arteritis, associated with migrating larvae in the heart and pulmonary vasculature. This study reports plasma coagulation variables for a larger population of *Otostrongylus*-affected seals, and uses TEG to provide a global measure of coagulation and fibrinolysis. To assess fibrinolytic variables, a tPA-modified assay was used. This technique accelerates fibrinolysis to facilitate measurement of lysis variables, and has been validated in people for detection of hyperfibrinolytic disorders.¹⁵ This modified assay has also been applied in dogs to measure fibrinolysis in cases of hemoperitoneum.¹⁹

In this study, affected seals were hypocoagulable on both PT and APTT and plasma TEG, with a consumptive process indicated by a decreased fibrinogen concentration and elevated D-dimers. These results are generally consistent with DIC. Vascular nematodes, including *Dirofilaria immitis*²⁶ and *Angiostrongylus vasorum*²⁷, have been associated with DIC in dogs, although in these cases the antigenic stimulus is an adult heartworm, as opposed to migrating larvae as seen in *Otostrongylus* arteritis.

While our data largely support a diagnosis of DIC, the platelet count in these NES was in the normal

range. Though case seals had a lower median platelet value compared to controls, this difference was not statistically significant and platelet count did not correlate with the presence of hemorrhage. Platelet activation in NES has been demonstrated to be relatively resistant to routine activators, including collagen and thrombin²⁸, and inadequate platelet activation or other thrombocytopathia may contribute to coagulopathy in the face of normal total platelet counts. Whole blood TEG analysis would be needed to assess the effect of platelet concentration on coagulation in *Otostrongylus* cases.

Alternatively, single time-point sampling may be causing us to overlook a downward trend in the platelet count of these animals. The ISTH recognizes 2 stages of DIC, overt and nonovert.⁵ Overt DIC describes the fulminant, hemorrhagic syndrome associated with a decompensated hemostatic system, while nonovert DIC describes a state of compensated hemostasis and risk of progression to overt DIC. A diagnosis of nonovert DIC includes serial sampling to document trends in platelet count, PT, and FDPs.⁵ Our data, relying on single samples, are insufficient to diagnose a state of nonovert DIC.

In contrast to our hypothesis of hyperfibrinolysis, *Otostrongylus*-positive seals were hypofibrinolytic compared to the reference population. Hypofibrinolysis is common in human patients with DIC secondary to infection or sepsis, mediated by activation of plasminogen activator inhibitor 1 (PAI-1).²⁹ A similar mechanism of PAI-1 activation may be present in cases of *Otostrongylus* arteritis, though preliminary measurement of PAI-1 in case seals did not support this hypothesis (data not presented). Hypofibrinolysis could also be attributed to plasminogen consumption, which has been reported in horses with DIC due to severe colic³⁰; however, plasminogen concentration in either healthy or *Otostrongylus*-positive seals is unknown. Medical research has also implicated endogenous carbon monoxide, a product of heme metabolism, as an antifibrinolytic mediator.³¹ Carbon monoxide interacts with heme groups on fibrinogen, plasmin, and α2-antiplasmin, leading to hypofibrinolysis.³¹ The degree of endogenous carbon monoxide production in NES is unknown. While the mechanism of hypofibrinolysis requires further research, the results of this study do not support the use of antifibrinolytic medications to treat cases of *Otostrongylus* arteritis, as previously investigated.⁸

There are several limitations to this study. Diagnosis of prepatent *Otostrongylus* arteritis is challenging, due to the lack of an antemortem tests for prepatent *Otostrongylus* infection and variable clinical presentations in affected NES. In this study, criteria used for initial diagnosis were variable, including both NES

with frank hemorrhage from an orifice, and seals with more nonspecific signs such as lethargy, anorexia, and regurgitation. To prevent misclassification errors, only seals with *Otostrongylus* found on gross necropsy were included in our analysis. However, this only identifies parasitic infection while histopathology is required for a definitive diagnosis of the arteritis syndrome. Seals with a very low worm burden may exhibit a severe arteritis and associated clinical signs, and in these cases, gross necropsy alone may be insufficient for a parasitic infection diagnosis.

An additional limitation is the use of anti-human D-dimer and AT antibodies in diagnostic assays, and comparison with a human plasma standard curve. Elephant seal-derived antibodies for assay development and calibration were not available, which prevented species-specific assay validation. Interpretation of our findings in clinically affected elephant seals is limited to comparison with a healthy reference population, tested with the same methodology.

This study was also limited by the use of plasma TEG, which does not account for the role of platelets, RBC and WBC, and the vascular endothelium on coagulation. Whole blood TEG would be informative, especially in light of the surprising platelet counts in these coagulopathic animals. However, whole blood TEG requires the sample to be analyzed within 30 minutes of collection which was not feasible in this study. Additionally, duplication of TEG assays as a method of quality control was employed for control seals, but could not be used in case seals due to limited volumes of available plasma. A review of TEG protocols suggests duplicating activated samples is unnecessary¹³, but it can be considered for use in exotic species when applying unvalidated protocols.

Validation of these assays for elephant seals will improve diagnostic utility and strengthen future research. Further investigation of alternative therapies for *Otostrongylus* arteritis, such as plasma transfusions or coagulation factor concentrates, is warranted.

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