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Authors: Leclerc, Lydie-Amy, Vergneau-Grosset, Claire, Fitzgerald, Guy, Brandão, João, and Gara-Boivin, Carolyn

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Original Study

Determination of Coagulation Parameters by Whole Blood Dynamic Viscoelastic Coagulometry in Strigiformes

Lydie-Amy Leclerc, Claire Vergneau-Grosset, Guy Fitzgerald, João Brandão, and Carolyn Gara-Boivin

Abstract: No reference values are available in Strigiformes to evaluate blood coagulation using dynamic viscoelastic coagulometry (DVC) with the Sonoclot (Sienco, Boulder, CO, USA) analyzer. The objectives of this study were 1) to assess the feasibility of DVC in Strigiformes, 2) to calculate the index of individuality of each coagulation parameter, and 3) to assess interspecies variability and establish reference intervals, if relevant, based on the index of individuality. Fresh whole blood samples were obtained from healthy Strigiformes, including 13 barred owls (*Strix varia*), 10 great horned owls (*Bubo virginianus*), 6 snowy owls (*Bubo scandiacus*), and 7 eastern screech owls (*Megascops asio*), and analyzed with DVC with glass bead (gb) and kaolin clay (k) coagulation activators. Activated clotting time (ACT), clot rate (CR), and platelet function were determined immediately after collection using fresh native whole blood. Intraindividual variability was assessed with a second fresh native whole blood sample from 5 barred owls. Interindividual variability was assessed using a Kruskall-Wallis test. For the parameters gbACT (n = 35), gbCR (n = 34), and kACT (n = 27), no significant differences were detected between species (all $P \geq 0.05$). Based on low index of individuality, global Strigiformes reference intervals were determined for gbACT (32.3–852.5 seconds; n = 35), gbCR (0–20.1 units/min; n = 29), and kACT (0–1570.3 seconds; n = 27). In conclusion, DVC can be used in Strigiformes and the gb coagulation activator would be more appropriate when basal individual values are not available in a tested individual.

Key words: Coagulation, viscoelastic, DVC, Sonoclot, bird of prey, Strigiformes, avian

INTRODUCTION

Hemostasis is a physiologic process essential to control bleeding following vascular damage. Coagulation assays developed in mammals have been used to asses coagulation in birds but some assays might not be adequate.[1] Avian coagulation is different from mammals because 2 coagulation factors in the intrinsic pathway, factors XI and XII, may be absent.[2,3] Bird coagulation appears to primarily rely on the extrinsic pathway, while the intrinsic pathway is believed to play a minor role.[4–6] Therefore, activated partial thromboplastin time, which evaluates the intrinsic pathway, is not suited for birds and may not be representative.[1] Prothrombin time (PT) can be used to evaluate the extrinsic and common pathways of coagulation in birds. However, there is no commercially available avian thromboplastin and extemporaneous solutions may not be homogenous or consistent between different aliquots.[4] Using a mammalian thromboplastin lengthens the PT.[5] Moreover, plasma-based tests such as PT and activated partial thromboplastin time do not integrate cellular components of coagulation and provide no information about the clot rate formation, the strength of the clot, or its dissolution.[1,7–9]

Viscoelastography, such as dynamic viscoelastic coagulometry (DVC), can be used in real time to measure the viscoelastic properties of the blood clot starting from the onset of its formation to its

From the Centre Hospitalier Universitaire Vétérinaire affiliated with the Faculté de Médecine Vétérinaire (Leclerc, Fitzgerald), the Département de Sciences Cliniques (Vergneau-Grosset), and the Département de Pathologie et Microbiologie (Gara-Boivin), Université de Montréal, 3200 Rue Sicotte, Saint-Hyacinthe, QC, Canada, J2S 2M2; and the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Oklahoma State University, 2065 West Farm Road, Stillwater, OK 74078, USA (Brandão).

Corresponding Author: Claire Vergneau-Grosset, claire.grosset@umontreal.ca

lysis, and can be used to determine the coagulation status of a given patient: normal, hypocoagulable, or hypercoagulable.[1,7–9] Dynamic viscoelastic coagulometry is currently used in human medicine to help manage massive bleeding, assess a patient's condition during cardiopulmonary bypass and liver transplantation surgery, and regulate heparin therapy.[9–11] In veterinary medicine, reference intervals for DVC have been previously established in healthy horses and dogs, and whole blood analysis using DVC is also feasible in healthy chickens.[1,7,8]

Birds of prey are exposed to anticoagulant rodenticides (AR) that pose a threat of intoxication to various species in North America. Numerous studies have detected one or more AR hepatic residues postmortem, although direct links between intoxication, hemorrhage, and mortality are difficult to evaluate, especially in the presence of trauma.[12–17] Little information is available about the sublethal effects of AR in birds of prey and antemortem assays to detect possible blood coagulation pathology secondary to AR intoxication are still under investigation. A study on brodifacoum toxicity in Japanese quail (*Coturnix japonica*) demonstrated a correlation between a prolonged PT and occurrence of hemorrhagic lesions at necropsy, but these lesions were not correlated with the ingested dose of brodifacoum nor the level of hepatic residues.[18] In addition, PT has been studied in 6 species of birds of prey in relation to detection of AR intoxication, but no reference intervals have been established for these species.[12] Detection of rodenticides in serum has been reported in red-tailed hawks (*Buteo jamaicensis*) but the method requires a large volume of blood collection (2 mL). This test would not be feasible for smaller avian species.[13] The objectives of the study reported here were to 1) assess the feasibility of DVC in Strigiformes, 2) calculate the index of individuality of each coagulation parameter, and 3) assess interspecies variability and establish reference values, if relevant, based on the index of individuality. The hypotheses of the study were 1) that DVC would be applicable in Strigiformes, 2) the calculated index of individuality would be below 1.7 for all measured parameters, allowing to establish reference values, and 3) that interspecies variability would be limited among Strigiformes

MATERIAL AND METHODS

Strigiformes included in this study were either captive (Chouette à voir, Saint-Jude, QC, Canada, and Aquarium du Québec, Quebec City, QC, Canada) or free-ranging birds undergoing rehabilitation at the Birds of Prey Clinic (Saint-Hyacinthe, QC, Canada, of the Université de Montréal). The study was approved by the Institutional Animal Care and Use Committee of the Faculté de médecine vétérinaire, Université de Montréal (21-Rech-2104).

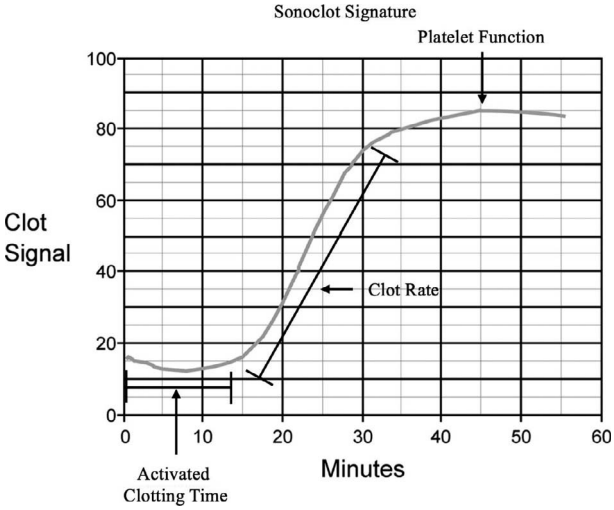

Figure 1. Example of Sonoclot signature with representation of activated clotting time, clot rate, and platelet function.

The inclusion criteria for the study were Strigiformes weighing more than 60 g, aged more than 6 months old, and who had received a controlled diet for at least 2 weeks. Exclusion criteria were Strigiformes who had received medication that could affect coagulation (eg, anti-inflammatory drugs) within the past 5 days. All birds were determined to be healthy based on physical examination and clinical follow-up during the research investigation. Included birds were housed in their respective institutions and were fed a diet consisting of commercial frozen-thawed mice, rats, and/or chicks.

In the case of DVC using the Sonoclot analyzer (Sienco, Boulder, CO, USA), whole blood is inserted into a fixed cuvette containing no additive or coagulation activator; a probe oscillates vertically in the sample during the analysis. During clot formation and subsequent lysis, the viscoelastic properties of the clot change with fibrin formation and create resistance to the probe's movement. The impedance is then converted via a transducer and reported in a curve (Sonoclot signature [SS]) (Fig 1). Three values can be obtained: activated clotting time (ACT), which is the time between initiation of the test and first fibrin formation; clot rate (CR), which represents the maximum speed of conver-

sion from fibrinogen to fibrin and is illustrated by the maximum slope of the SS; and platelet function (PF), which is a calculated value in relation to time and quality of clot retraction.[9] A single dual-chamber DVC (Sonoclot, SCP2 model, Sienco) was used to determine ACT, CR, and PF coagulation parameters and SS using 2 coagulation activators: glass beads (gb) and kaolin clay (k). The gbACT+ and kACT settings were used for the gb and k coagulation activators, respectively. The sample was automatically mixed for approximately 10 seconds and heated to 37°C (98.6°F) prior to analysis. All analyses were performed by 2 trained operators (L.A.L. and C.V.G.). Routine maintenance and quality control procedures were performed in accordance to the manufacturer's recommendations. Calibration with a reference viscosity standard was completed daily before any testing, and room temperature was kept within the interval limits specified by the manufacturer.

For each bird, 0.6 mL of whole native blood was collected from the jugular, ulnar, or medial metatarsal vein with a 1-mL syringe and a 25-gauge needle (Terumo Corporation, Binan, Laguna, Philippines). The birds were not sedated for blood collection. The same venipuncture site was attempted for all individuals of a given species but an alternate venipuncture site was used in case of hematoma formation. The whole native blood was divided from the syringe directly into 2 cuvettes heated to 37°C containing each coagulation activator; gb first, followed by k, and run within 1 minute for analysis. Analysis ended when ACT, CR, and PF (gbACT+ setting only) values were obtained or after a 40-minute time limit. To determine intraindividual variability, blood was collected a second time from 5 barred owls (*Strix varia*) at least 2 weeks after the first sample collection and processed using the previously described methods.

The index of individuality was calculated in Microsoft Excel using the following formula:

$$\frac{CV_G}{\sqrt{CV_I^2 + CV_A^2}},$$

where CV_G represents the variance between individuals, CV_I is the variance within individuals, and CV_A is the analytical variation.[19] In this study, the analytical variation was not evaluated. Intra-assay coefficient of variation (CV) was determined in healthy chickens with fresh whole blood for ACT (6.85%) and CR (11.3%).[1] When the index of individuality was $\leq$1.7, reference intervals were calculated for the corresponding parameter.

Reference values were described for barred owls and great horned owls (*Bubo virginianus*) for parameters with $10 \leq x < 20$ samples in accordance with the American Society for Veterinary Clinical Pathology guidelines for zoological species.[20] Statistical analysis was performed by R (version 4.0.5, R Foundation for Statistical Computing, Vienna, Austria). Coagulation parameters were evaluated for normality using the Shapiro-Wilk test. The Kruskal-Wallis test was used to determine if differences between species were significant. Paired Wilcoxon tests were used to determine if differences between blood coagulation activators were significant. Values of $P < 0.05$ were considered statistically significant. The software Prism (version 9.3.1, GraphPad Software, San Diego, CA, USA) was used to create box plots. General reference intervals for all 4 species were established for parameters with an index of individuality below 1.7 by the Reference Value Advisor Software (Greffé *et al*. 2011; École Nationale Vétérinaire de Toulouse, 2018).[21] For data normality assessment, a P value of 0.19 was used as previously described for small datasets including less than 40 individuals.[22] Outliers were excluded using Tukey method when data were normally distributed and graphically for non-normal distributions. The Box-Cox transformation standard method was used to establish activated clotting time (gbACT) and clot rate (gbCR) using gb activator reference intervals. The robust method was used to establish activated clotting time using k activator (kACT) reference intervals. When a robust method was used, symmetry testing was confirmed to have a $P > 0.05$.

RESULTS

Thirty-six Strigiformes were included in the study from 4 different species: 13 barred owls, 10 great horned owls, 6 snowy owls (*Bubo scandiacus*), and 7 eastern screech owls (*Megascops asio*) (Table 1). Among birds included in the study, 5 barred owls, 5 great horned owls, 2 snowy owls, and 2 eastern screech owls were captive, while the remaining individuals were free-ranging and being rehabilitated. Blood was collected consistently from the jugular vein in barred owls and from the ulnar vein in snowy owls. In great horned owls, 6 blood samples were collected from the jugular vein, 3 from the ulnar vein, and 1 from the medial metatarsal vein. In eastern screech owls, 6 blood samples were collected from the jugular vein and 1 from the ulnar vein. Hematoma formation at the initial venipuncture site was present in 4 birds: 1

Table 1. Distribution of the sexes of birds included in a study about establishment of coagulation parameters by whole blood dynamic viscoelastic coagulometry in Strigiformes.

Common name	Species	Male	Female	Unknown sex	Total
Barred owl	*Strix varia*	3	3	7	13
Great horned owl	*Bubo virginianus*	2	3	5	10
Snowy owl	*Bubo scandiacus*	1	3	2	6
Eastern screech owl	*Megascops asio*	2	1	4	7

barred owl and 1 great horned owl had hematoma formation in the right jugular vein and 1 great horned owl and 1 screech owl had hematoma formation in the left ulnar vein. In these cases, an alternative venipuncture site was used to ensure no hematoma formation at the final sample site. Those last samples (ie, collected from animals with hematomas) were run for analysis and each yielded a result. All blood collections included in the study were collected within 10 seconds of venipuncture and run for Sonoclot analysis within a minute. For 2/13 (15.4%) barred owls, 3/10 (30%) great horned owls, 1/6 (16.7%) snowy owls, and 2/7 (11.7%) eastern screech owls, values with k blood coagulation activator were not determined due to analyzer error or the analysis taking more than 40 minutes. The results for each species for gbACT, kACT, gbCR, and CR with k activator (kCR) are presented in Figure 2. The results of PF with gb activator (gbPF) are presented in Figure 3. In comparison, kACT tended to be longer than gbACT in all species but the differences were not significant. Results for kCR were generally lower than gbCR in all species and the difference was significant ($P < 0.0001$), which means that the

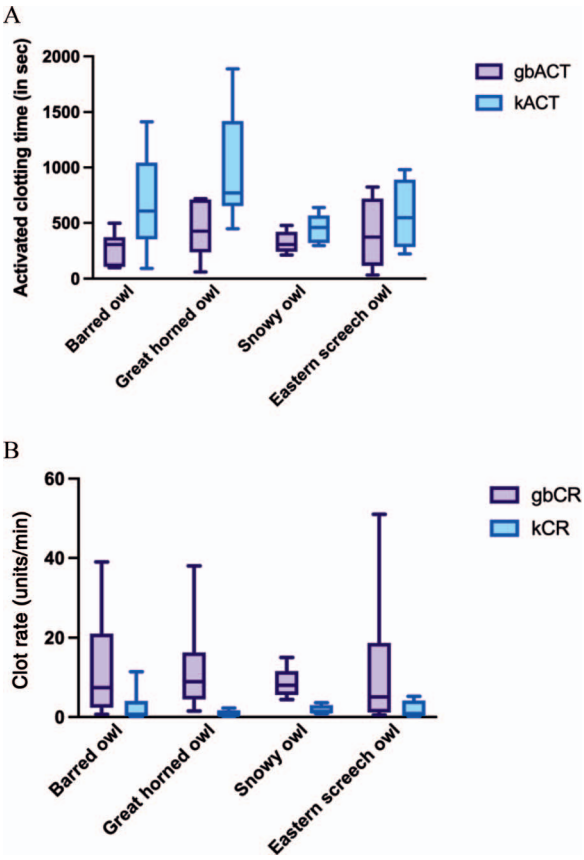

Figure 2. Coagulation parameters measured in barred owls (*Strix varia*), eastern screech owls (*Megascops asio*), great-horned owls (*Bubo virginianus*), and snowy owls (*Bubo scandiacus*) using a glass-bead blood coagulation activator (gbACT) or a kaolin clay blood coagulation activator (kACT): (A) Activated clotting time in seconds, (B) clot rate (unit/min). Each box represents the first to third quartiles (interquartile [25th to 75th percentile] range), the whiskers represent the minimum and maximum values, and the horizontal line in each box represents the median.

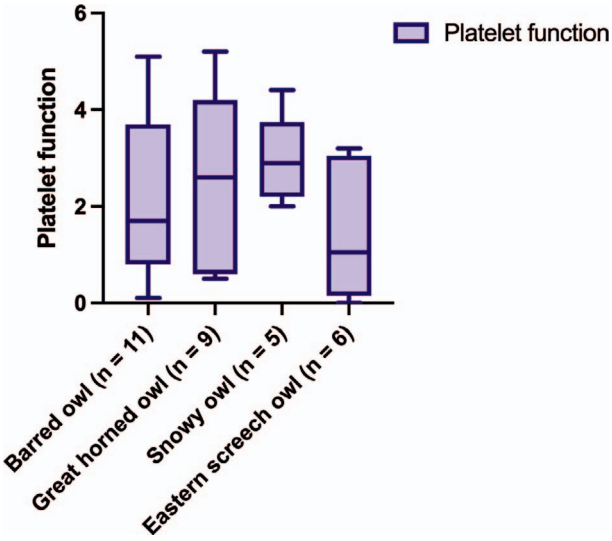

Figure 3. Platelet function measured in barred owls (*Strix varia*), eastern screech owls (*Megascops asio*), great-horned owls (*Bubo virginianus*), and snowy owls (*Bubo scandiacus*) using a glass-bead blood coagulation activator. Each box represents the first to third quartiles (interquartile [25th to 75th percentile] range), the whiskers represent the minimum and maximum values, and the horizontal line in each box represents the median.

Table 2. Indices of individuality for each of the dynamic viscoelastic coagulometry coagulation parameters established in Strigiformes.

Parameters	Index of individuality
gbACT	1.28
gbCR	0.39
gbPF	2.36
kACT	1.20
kCR	4.02

Abbreviations: gbACT, glass-bead activated clotting time; gbCR, glass-bead clot rate; gbPF, glass-bead platelet function; kACT, kaolin clay activated clotting time; kCR, kaolin clot rate.

speed of conversion from fibrinogen to fibrin is faster with the gb activator.

Indices of individuality were calculated for gbACT, gbCR, gbPF, kACT, and kCR (Table 2). Based on these results, references values were established in barred owls for gbACT, gbCR, and kACT (Table 3) and in great horned owls for gbACT and gbCR (Table 4). There were no statistical differences between the 4 species of the study for gbACT ($P = 0.34$; n= 35), gbCR ($P = 0.70$; n = 34), and kACT ($P = 0.12$; n = 27). Thus, global reference intervals were calculated in Strigiformes for gbACT, gbCR, and kACT (Fig 4, Table 5). The reference interval in Strigiformes for gbACT was 32.3 to 852.5 seconds (n = 35) and no outliers were excluded. The reference interval in Strigiformes for gbCR was 0 to 20.1 units/min (n = 29) after exclusion of 5 outliers. The reference interval in Strigiformes for kACT was 0 to 1570.3 seconds (n = 27) and no outliers were excluded.

DISCUSSION

Results reported in this study support that DVC is feasible in 4 Strigiformes species using gb and k blood coagulation activators, which both act as a negative charge surface to initiate coagulation and could be used as a coagulation assay.[9] Despite the belief that the intrinsic pathway plays a minor role in birds' coagulation, our study demonstrated that it is possible to initiate coagulation via the contact pathway in Strigiformes when an proper activator (eg, gb or k) is used.[4–6] In chickens, it was also possible to use gb and k blood coagulation activators to initiate coagulation and yield results.[1] Analysis using the k coagulation activator was more prolonged (ie, up to 35 minutes) in comparison to gb. Moreover, the k coagulation activator did not yield kACT and kCR values consistently because of analyzer error. The lack of results suggests that the k activator may not be as potent as gb in Strigiformes. In chickens, results had been obtained for each individual using the k activator, and the median kACT was 6 minutes in chickens versus 10 minutes in Strigiformes. A study using thromboelastography showed that activation of the intrinsic pathway using the k activator yielded similar results compared to whole citrated blood without activator.[6] The order of cuvette filling used in the present study, with k being the latter test, might have influenced the result because this test was performed a few seconds later than the assay with gb. However, assays were run within a minute from venipuncture. If coagulation had been initiated in the syringe prior to analysis, coagulation would have been expected be quicker with k, which was not the case in our study.[8] Also, SS graphics were similar to other species and did not display aberrant patterns that would imply the blood sample was inadequate.

According to the index of variability, interpretation of kCR ($\geq$ 1.7) should be based on individual basal value. In a context of rehabilitation, this is impractical because admitted patients might already have a coagulation abnormality and assessing individual basal value is not possible in free-ranging birds. For these reasons, the gb coagulation activator may be better when evaluating blood coagulation status in Strigiformes. The use of a DVC device presents many advantages. It gives quantitative and qualitative results to assess the blood coagulation process that are easy to interpret, requires a small volume of blood for

Table 3. Reference values and medians for activated clotting time using a glass-bead blood coagulation activator, clot rate using a glass-bead blood coagulation activator, and activated clotting time using a kaolin clay blood coagulation activator in barred owls (*Strix varia*).

Parameter	Reference value	Median	n
gbACT, s	99–498	306	13
gbCR, units/min	0.7–39	7.4	13
kACT, s	93–1413	607	11

Abbreviations: s, seconds; min, minutes; n, number; gbACT, glass-bead activated clotting time; gbCR, glass-bead clot rate; kACT, kaolin clay activated clotting time.

Table 4. Reference values and medians for activated clotting time and clot rate using a glass-bead coagulation activator in great horned owls (*Bubo virginianus*).

Parameter	Reference value	Median	n
gbACT, s	60–719	425	10
gbCR, units/min	1.5–38	12.7	10

Abbreviations: s, seconds; min, minutes; n, number; gbACT, glass-bead activated clotting time; gbCR, glass-bead clot rate.

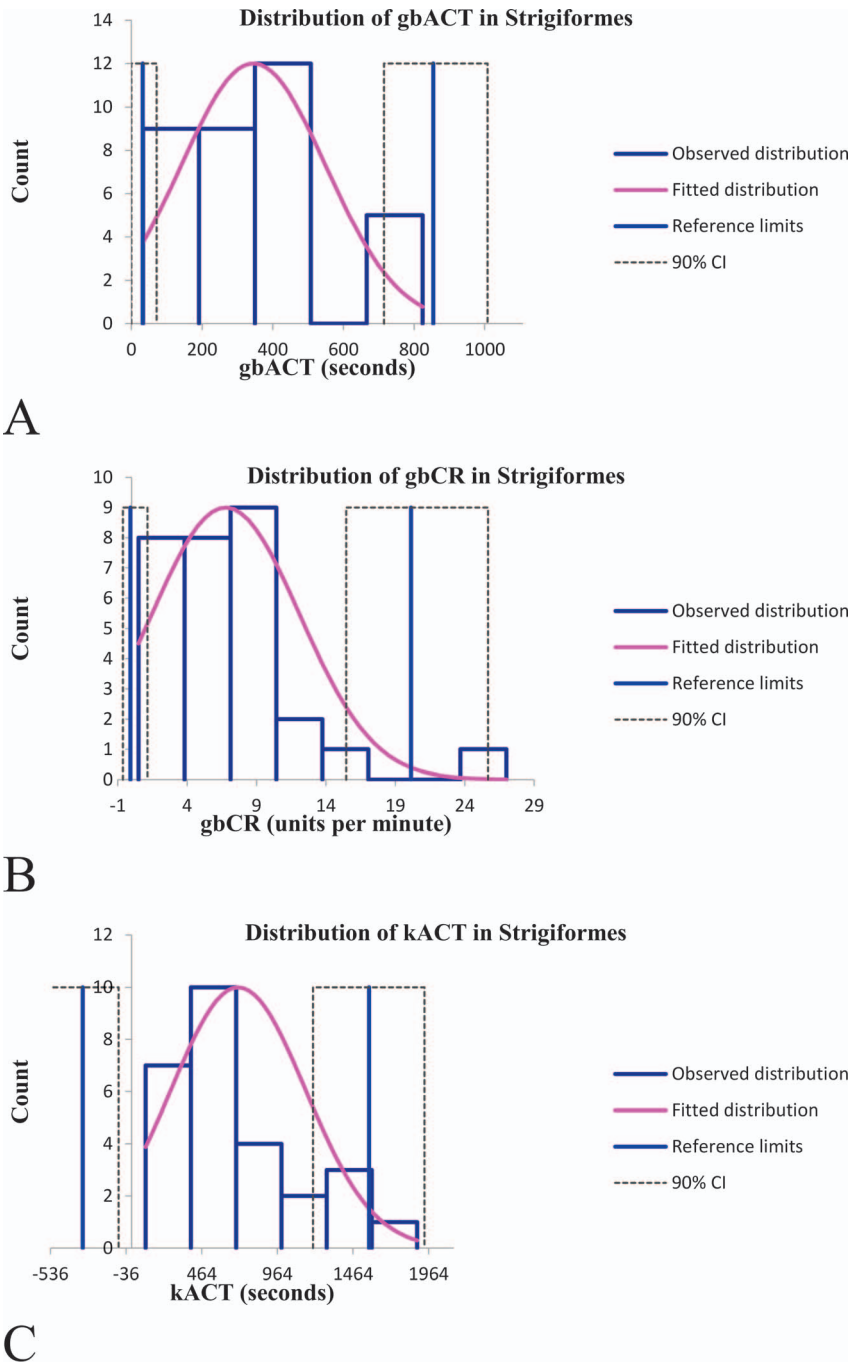

Figure 4. Each figure represents the distributions of the data for the 3 parameters (A, glass-bead activated clotting time; B, glass-bead clot rate; C, kaolin clay activated clotting time) used to generate the reference intervals, with the number of birds on the Y-axis, included in each class of the histogram.

analysis, offers a quick turnaround time, and is less expensive than other viscoelastic coagulation assay analyzers.[7,9] Operator training and device maintenance is uncomplicated, and the DVC can be easily used in various laboratories and clinics. Unfortunately, there is no standard method using DVC in animals and protocols should take into account rest time period and agitation in their design.[8,9] Whole native blood instead of citrated recalcified blood was used in this study because intra-assay CV values were close to or below 10% in chickens.[1] To use whole native blood in a clinical context, the DVC device must be readily available and close at hand, as the time required between blood collec-

Table 5. Reference intervals for activated clotting time (n = 35) and clot rate (n = 29) using a glass-bead blood coagulation activator and activated clotting time using a kaolin clay blood coagulation activator (n = 27) in Strigiformes following the American Society for Veterinary Clinical Pathology guidelines.

Parameter	Reference intervals	Median	Range	n	90% CI (lower limit)	90% CI (upper limit)	Statistical analysis method
gbACT, s	32.8–852.5	322	33–824	35	0–19.3	666.3–864.6	Box-Cox transformation with standard technique
gbCR, units/min	0–20.1	6.7	0.5–27	29	0–1	15. 2–25.5	Box-Cox transformation with standard technique
kACT, s	0–1570.3	624	93–1888	27	0–0	1223.7–1897.7	Robust technique

Abbreviations: s, seconds; min, minutes; n, number; CI, confidence interval; gbACT, glass-bead activated clotting time; gbCR, glass-bead clot rate; kACT, kaolin clay activated clotting time.

tion and analysis is <2 minutes. Further studies will be necessary to establish a standardized protocol for DVC use in birds. The index of individuality is a tool that can be used to determine whether results should be interpreted according to a reference interval or an individual baseline, based on a ratio of interindividual to intraindividual variability. When the index of individuality is ≤0.7, reference intervals should be used to interpret results. When the index of individuality has a value between <0.7 and >1.7, reference intervals or basal individual values could be used to interpret results. When the index of individuality has a value ≥1.7 basal, individual values should be used to interpret results.[19] Index of individuality were successfully calculated and showed that interpretation using reference intervals is appropriate for gbACT, gbCR, and kACT (<1.7). Conversely, interpretation of gbPF and kCR should be based on basal individual values and reference intervals were not relevant (≥1.7). Reference values were established for gbACT, gbCR, and kACT in barred owls and gbACT and gbCR in great horned owls and can be use in clinical practice to assess coagulation status of these birds. In addition, global references intervals for gbACT, gbCR, and kACT have been calculated in Strigiformes. The median gbACT value in Strigiformes (322 seconds) is similar to the median values published in healthy horses and chickens (308.4 and 338 seconds, respectively).[1,8] The median gbCR in Strigiformes (6.7 units/min) is lower than in horses (10.49 units/min) and chickens (13.9 units/min).[1,8] The median kACT in Strigiformes (624 seconds) is substantially higher than the median in chickens (353 seconds).[1] To best interpret avian patients' coagulation status using the DVC device, order-specific or species-specific references intervals should ideally be established given the differences noted between chickens and Strigiformes.

The present study carried some limitations. This study included a limited number of Strigiformes of each species, which precluded calculation of reference intervals in snowy and screech owls. Further studies including a greater number of birds would be necessary to establish reference intervals for the Strigiformes order. For each parameter, Strigiformes presented a wide range and large reference intervals, illustrating a high interindividual variability. High interindividual variability has also been described in healthy horses.[8] Preanalytical factors might have influenced the results of the present study. The effect of sex and age was not evaluated in the present study, but might have contributed to the high interindividual variability. Reference intervals were established with combination of all 4 species included in this study. Specific differences between Strigiformes species might have influenced results and contributed to large reference intervals. In this study, a significant difference was not detected between species using the Kruskal-Wallis test, but a larger population of birds might reveal statistical differences. A study in cats showed significant differences in viscoelastic parameters during clot formation between jugular and saphenous venipunctures using a distinct coagulation testing device.[23] In our study, the venipuncture site was consistent in barred and snowy owls but not in great horned or eastern screech owls due to technical difficulties. This might have influenced the results in these 2 species. However, barred owls displayed wide interindividual variability despite a consistent venipuncture site. In 4 birds, hematoma formation was present at the initial venipuncture site, 2 in the right jugular vein and 2 in the left ulnar vein, thus an alternative site without hematoma formation was used for analysis. If clot formation was occurring at the initial venipuncture site, coagulation factors may have been in blood circulation while collecting the alternative site and may have influenced the

result by a faster clot formation. The gbCR value of 1 screech owl with hematoma formation in the left ulnar vein was an outlier with a faster clot formation, while the 3 other birds with hematoma formation had a similar clot formation profile to other individuals, with no overall trend towards a faster or slower clot formation. Thus, the authors chose to include these three birds in the Strigiformes reference interval. In addition, birds included in the study were considered healthy based on physical examination and clinical monitoring, but complementary tests such as complete blood counts and plasma biochemistry panels were not performed to assess health status. Subclinical disease, such as hepatic disease, thrombocytopenia, inflammation, anemia, or infection process, if present, might have influenced the results. Of the18 birds included in the study that had previous packed cell volume (PCV) assessment, 14/18 (77.8%) birds had normal PCV and 4/18 (22.2%) birds had a PCV value of 1% below or above reference intervals.[24] A thrombocyte count was not determined prior to DVC analysis on any bird included in this study. Blood coagulation requires thrombocyte activation to form the clot and abnormalities in PCV or the thrombocyte count may affect viscoelastic results.[25] However, a study that used Sonoclot in dogs showed no significant correlation between Sonoclot parameters and PCV and did not reveal a correlation between CR and platelet count, as opposed to what is reported in human medicine where a positive correlation was detected between CR and platelet count.[7,11] In dogs, a weak positive correlation was present between platelet count and PF.[7] Free-ranging rehabilitated Strigiformes included in the present study were assumed to be free of AR intoxication following 2 weeks of strict diet and a lack of clinical signs of coagulopathy. Japanese quail and American kestrels (*Falco sparverius*) experimentally intoxicated with diphacinone showed signs of toxicity within 48 hours of exposure.[15] Even if detection of AR intoxication was not performed in our study, exposure was considered very unlikely. Effect of body temperature and the temperature of the analysis may also be relevant. Blood samples were heated to 37°C by the DVC, which is the human body temperature; avian species have a higher body temperature. This might have influenced the results since a lower temperature might lengthen the reaction time, leading to relative hypocoagulation.[9] This temperature was chosen based on previous studies in chickens, but further studies may evaluate coagulation parameters at higher temperatures. Intraoperator variability was not calculated, but DVC analysis is known to have a better intraoperator reliability in comparison with thromboelastography.[9] In addition, this study did not use avian thromboplastin activator because it is not commercially available. A study in horses that used tissue factor as an activator showed less interindividual variability compared to no additive. The use of a stronger activator, such as homogenous avian thromboplastin, might help reduce interindividual variability and tighten reference intervals.[25] Further studies should establish reference intervals for DVC parameters using avian thromboplastin as described in reptiles.[26] Finally, reference intervals reported in this study should be used cautiously because institutional reference should be calculated for the individual laboratory and specific analyzer used.

In conclusion, DVC can be used in Strigiformes and reference intervals were established for gbACT, gbCR, and kACT. Using the gb coagulation activator would be more appropriate when basal individual values are not available in a patient. Further studies that include a greater number of birds within the study groups and birds that are experimentally intoxicated with AR are necessary to evaluate if hypocoagulation secondary to AR intoxication in Strigiformes can be detected with the established references intervals.

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