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ARTICLE

Reproducibility of blood lead levels after blood stored for seven days in trumpeter swans (*Cygnus buccinator*) and mute swans (*Cygnus olor*)

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Abstract

Wild animals, particularly birds, often present with clinical signs of lead toxicosis when admitted to wildlife rehabilitation centers. Many wildlife rehabilitators and wildlife biologists do not have a point-of-care blood lead level analyzer, nor do they always have the financial resources to submit samples for external laboratory analyses. Often there is a delay between taking a blood sample in the field and subsequent analysis. The objective of this study was to determine whether a delay in processing a blood sample for lead testing would significantly alter the blood lead level results in swans. Whole blood samples were collected from 49 trumpeter swans and four mute swans and placed in ethylenediaminetetraacetic acid (EDTA) blood collection tubes. Samples were run on a LeadCare II analyzer at day 0, stored at 4°C for 7 days, and rerun on day 7. Results indicate that whole blood can be stored in EDTA blood collection tubes for up to seven days at 4°C while maintaining reproducibility in blood lead level results that were analyzed from a point-of-care blood lead level system.

Keywords

Blood lead level; lead analysis; lead toxicosis; trumpeter swan

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Abbreviations

C: Celsius
EDTA: ethylenediaminetetraacetic acid
GFAAS: graphite furnace atomic absorption spectroscopy

Dates

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BIO

Dr. Sherri Cox is a wildlife veterinarian and the medical director of the National Wildlife Centre Canada. As a board-certified specialist (ABVP, Avian), she and her medical team provide medical oversight for wildlife patients at four wildlife rehabilitation centers in Canada. Sherri holds a PhD from the University of Guelph where her research is focused on lead toxicosis in free-ranging trumpeter swans in Ontario, Canada.

Introduction

Lead toxicosis has been well documented in wild animals and causes significant mortality among wildlife, particularly waterfowl. The most common cause of death in the trumpeter swan reintroduction program in Ontario, Canada, was lead poisoning, caused by ingestion of lead shot or fishing sinkers (Lumsden & Dreve 2002). Approximately 80% (1375/1727) of trumpeter swan deaths in the Pacific Northwest were attributed to ingestion of lead shot (Wilson et al. 2009). Nearly 10 000 swans comprising six species have died from poisoning caused by lead that originated from ingestion of fishing weights, shotgun pellets, or contaminated sediment (Blus 1994). In addition, an estimated 125 to 187 million lead sinkers are deposited in Canadian waters every year. Approximately 57% of adult common loons (*Gavia immer*) collected from freshwater died from lead toxicosis (Pokras & Chafel 1992). Mortality from lead toxicosis is likely under-reported as many birds are not found nor are tested (Twiss & Thomas 1998).

In one study of cattle exposed to lead, whole blood was taken in heparinized Vacutainers© and subsequently refrigerated for up to 72 hr before analysis with the LeadCare II System was complete. Results indicated this was acceptable for clinical purposes (Bischoff et al. 2010).

Most birds that ingest lead can become subclinically or clinically affected by the toxic effects lead has on the body (Pain et al. 2019). Those monitoring populations of wild birds or clinically treating wild animals are often at a disadvantage if they are unable to analyze blood lead levels in a timely manner. Not every wildlife rehabilitation center or veterinary hospital has immediate access to a blood lead level analyzer.

Wild animals, particularly birds, often present with clinical signs of lead toxicosis when admitted to wildlife rehabilitation centers. Many wildlife rehabilitators and wildlife biologists do not have a point-of-care blood lead level analyzer, nor the financial resources, to submit samples for external laboratory analyses immediately. In some cases, the time between collection sampling and blood

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analysis for the presence of lead may be delayed while whole blood is sent to another wildlife center or laboratory where a point-of-care blood lead analyzer is available and accessible. This delay could lead to inaccurate results as they were not obtained according to the manufacturer's instructions. Further, inaccuracies in results could change when and how animals are treated for possible lead toxicosis as well as the decision of whether to euthanize or release the animal.

The objective of this research is to evaluate the reproducibility of blood lead levels after blood has been collected in an EDTA blood collection tube and stored at 4°C for seven days. This information will be helpful to wildlife rehabilitators, wildlife veterinarians, biologists, and others working in the field where it is not always possible to analyze blood within 24 hr.

The most sensitive method for analysis of lead in blood or the liver is atomic absorption spectrophotometry (Degernes et al. 1989; Redig & Arent 2008). Whole blood is sent to a lab; results can take one to three days, making an in-house lab analyzer more practical with faster results. The point-of-care blood lead analysis systems (LeadCare II, Meridian Bioscience, OH) rely on electrochemistry and a sensor to detect lead concentration in whole blood, particularly the red blood cells. According to the manufacturer, the accuracy characteristics of the LeadCare II system suggest the following bias (Table 1) based on results run by GFAAS (Meridian Bioscience 2020). This accuracy was determined by comparing samples run on six LeadCare II Analyzers over five days with results analyzed by GFAAS.

The LeadCare II System is commonly found in larger wildlife rehabilitation centers and in select veterinary hospitals that treat wildlife. Currently, the LeadCare II System requires whole blood to be evaluated within 24 hr or seven days if stored in the provided treatment reagent tube and subsequently refrigerated. Treatment reagent tubes have expiration dates and must match the lot numbers of the diagnostic sample kit used, therefore the reagent tubes cannot be stored or distributed for sampling making blood collection a more viable option for subsequent analysis.

In the United States, federal regulations allow laboratories that perform blood lead testing to operate with a total allowable error of ±4 µg/dL or ±10%, whichever is greater (CDC 2007). However, there are no published error values in avian species, nor has the LeadCare II System been approved for veterinary use, although the analysis of blood lead levels is a common practice in veterinary medicine, specifically in wildlife health.

This research compares differences in blood lead levels analyzed at day 0 and again at day 7 from trumpeter swans (*Cygnus buccinator*) and mute swans (*Cygnus olor*) against the allowable error values currently in place through federal regulations for use in humans.

Table 1 Manufacturer reported average bias of LeadCare II analyzer compared to GFAAS.

Lead Concentration (µg/dL)	LeadCare II Bias from GFAAS	LeadCare II % Bias from GFAAS
0–10	0.07	-
10.1–25.0	-	4.7%
25.1–65	-	5.0%

Methods

Blood was collected from 49 trumpeter swans and four mute swans in Ontario, Canada, and placed into EDTA blood collection tubes. The majority of blood collected was from free-ranging swans (46/49) with 3/49 samples from birds in rehabilitation. This protocol was approved by the Animal Care Committee at the University of Guelph, AUP#4168.

Blood lead levels were tested using an in-house lead analyzer (LeadCare II System). Instructions were carefully followed according to the recommendations provided by the manufacturer. Specifically, 50 µL of whole blood placed into an EDTA blood collection tube was analyzed within 24 hr when stored between 10°C and 32°C, and 50 µL of whole blood taken from an EDTA blood collection tube was placed into the provided reagent vial, refrigerated, and analyzed at seven days post collection. Handling and temperature control were per the manufacturer's recommendation, and quality control procedures were implemented with each new lot of reagents.

Whole blood samples were refrigerated at 4°C in an EDTA blood collection tube for seven days from initial collection, at which time the lead test was run again for comparison. Levels that read "low" were assigned a value of 3.2 µg/dL based on the manufacturer's directions. whereby "low" indicates a blood lead test result of less than 3.3 µg/dL.

Statistical analysis

A paired samples t-test between day 0 blood lead concentration and day 7 blood lead concentration was conducted in IBM SPSS 26 to determine whether the two measures were statistically different. One sample *t*-tests were used to evaluate whether the differences between day 0 and day 7 were different than the recommended values of ±4 µg/dL and ±10%.

Table 2 Range, mean, difference and percent difference for 49 samples at day 0 and day 7.

	Day 0 µg/dL	Day 7 µg/dL	Difference µg/dL ± Std. Dev.	Percent Difference ± Std. Dev.?
Range	3.2–39.5	3.2–39.6	1.03 ± 0.32	8.43 ± 3.54
Mean	10.9 ± 1.16	9.9 ± 1.15	-	-

Results

The paired samples *t*-test showed that there was a significant difference between the blood level concentrations taken on day 0 and those taken on day 7 ($p = 0.003$). Table 2 shows the mean and ranges of the blood lead concentrations.

Two one-sample *t*-tests were conducted. The first was used to evaluate whether the difference between day 0 and day 7 differed from the recommended ±4 µg/dL. The average difference in this dataset was 1.03 µg/dL, which was statistically different than the recommended 4 µg/dL) $p < 0.0001$). The second one-sample *t*-test was used to test the recommended value of ±10% difference between day 0 and day 7. The mean difference between day 0 and day 7 (1.03 or 8.43%) was compared to the standard value of 10%. It was determined that the value of 8.43% was not different from the 10%. Therefore, these results were within the recommended range.

Discussion

In this study, reproducibility of results with more accessible storage conditions is critically important to determine the potential prognosis for return to health, the decision of whether to treat the animal, or the disposition recommendation to release or euthanize the animal depending on laboratory analysis results. Based on the current manufacturer's recommendations of the LeadCare II System, whole blood must be analyzed within 24 hr or placed into a reagent vial and refrigerated for up to seven days. If a researcher, wildlife rehabilitator, or wildlife veterinarian does not have a point-of-care analyzer, they must currently send to an external lab immediately or find a center that can analyze the blood within the given timeframe and parameters. This is not always possible for many people interested in studying, monitoring, or treating lead toxicity in wildlife.

In this study, two samples were deemed to be outliers and excluded from the results above due to the vast difference between the samples at day 0 and day 7. The difference was most likely due to human error in recording an incorrect value or a mislabelled blood collection tube.

The results of this study suggest that whole blood for trumpeter swans and mute swans could be stored in an EDTA blood collection tube and refrigerated at 4 degrees Celsius for up to seven days with reproducible results with clinical relevance. While there was a statistically significant difference between day 0 and day 7 results, this difference was within acceptable laboratory error. While this study only looked at trumpeter swans and a small sample of mute swans, it may be possible to extrapolate this storage time to other avian species.

Clinical relevance as well as reproducibility is important. Values > 20 µg/dL can be clinically significant in birds (Samour & Naldo 2002) and the clinical relevance of delaying treatment based on the inability to obtain and analyze a blood sample in a timely manner would be inappropriate as medical care, including chelation therapy and removal of lead may need to be initiated as soon as possible to minimize the potentially irreversible toxicity. Therefore, if the goal is to restore the health of the animal, appropriate treatment can be initiated in the interim for clinically ill birds while waiting for blood lead level results that could then change prognosis, treatment, and/or disposition decisions.

Conclusion

Whole blood from trumpeter and mute swans can be stored in an EDTA blood collection tube for up to seven days at 4°C while maintaining clinically adequate reproducibility in blood lead level results that are to be analyzed from a point-of-care blood lead level system.

This research could benefit wildlife rehabilitators, wildlife veterinarians, and wildlife biologists working in the field who may not have immediate access to a blood lead level analyzer.

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