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LETTER TO THE EDITOR

Dear Editor:

We write in reference to the article published in JZWM "Serum ferritin concentration is not a reliable biomarker of iron overload disorder [IOD] progression or hemochromatosis in the Sumatran rhinoceros (*Dicerorhinus sumatrensis*)."[16] We propose an alternative perspective that highlights the utility of the Roth et al. assay[16] as part of prevention and treatment of IOD.

We commend the authors for developing a rhino-specific ferritin assay, which, based on the data presented, appears more sensitive than the commonly used horse spleen ferritin assay[18] at detecting Sumatran rhino ferritin. The authors conclude "neither a specific pattern nor the individual's overall mean ferritin concentration can be used to accurately assess IOD progression" and "serum ferritin concentration does not appear to be very informative in some susceptible species."[16] This conclusion seems at odds with the data presented from three rhinos that died during the study interval. These animals had increasing serum ferritin over time, and high postmortem liver iron (~10,000–26,000 μg/g). Hemochromatosis was listed as the cause of death for two animals; the other animal reportedly died of thyroid cancer. The upper limit of a safe hepatic iron level in rhinos is unknown; however, in humans, hepatic iron >8,000 μg/g is associated with increased risk of life-threatening, iron-related complications.[1,6,13,19] Although there did not appear to be a correlation between mean serum ferritin and liver iron concentrations in this small data set, the pattern of consistently elevated and progressively increasing serum ferritin within nonpregnant individuals is suggestive of IOD or inflammatory disease[2,8] and consistent with high liver iron concentrations postmortem. Adult cases with low serum ferritin or liver iron would serve as negative controls to test correlative relationships. The profile of a reproductive female with decreasing and increasing serum ferritin concomitant with gestation and parturition, respectively, provides an additional biological validation of the ferritin assay; pregnancy is well established as one of the most efficient ways to remove iron stores.[5] Despite reductions in iron load during pregnancy, this female had elevated serum ferritin when not pregnant and antemortem, which were consistent with markedly high liver iron concentrations postmortem. In the future, using a published tissue scoring system[20] in all liver lobes[4] and other iron-loading iron tissues (e.g., lung)[10,12,14] would better characterize the extent of pathologic iron deposition postmortem and elucidate the relationship between serum ferritin and total body iron burden.

As ferritin is an acute-phase protein and can be elevated in the absence of IOD, we agree with Roth et al. that it should not be used in isolation as a definitive diagnosis of IOD in mammals, including humans.[21] Ideally, serum ferritin would be measured in tandem with iron-independent biomarkers of inflammation to distinguish between IOD and inflammatory conditions. Assays for several inflammatory biomarkers have been validated in black rhino serum; however, values may have been confounded with IOD in individuals tested.[17] Until specific biomarkers independent of iron status for rhinos are identified, the combination of repeatedly high or increasing serum ferritin concentration and elevated (>45–50%) percent transferrin saturation is the first-line and currently the most efficient approach to screen for IOD, though not necessarily to predict morbidity onset.[1,3,9,15]

Roth et al. found higher concentrations of ferritin singly measured in Malaysian-held rhinos compared to U.S. zoo rhinos "surprising" given previous reports implicating reduced browse diversity and increased iron in U.S. rhino diets with high ferritin.[16] The inclusion of an iron-laden mineral block in Sumatran rhino diets[7] and potential differential consumption, however, may have muddied comparisons among individuals and could account for why serum ferritin concentrations did not align with the authors' expectations. Given that browsing rhinos under managed care are predisposed to IOD,[14] continued efforts should be made to avoid feeding an iron-rich diet, including mineral blocks.

Until more specific biomarkers of IOD in rhinos are validated, we do not recommend discarding the tools we have in hand. The research and assay development by Roth et al. are valuable additions to our knowledge base optimizing rhino health *ex situ*. Excessive iron load in rhinos, as is the case in any mammal, increases the risk of many diseases and compounds negative health consequences.[11] Monitoring both serum ferritin and transferrin saturation routinely in browsing rhinos remains essential for comprehensive medical care. This recommendation complements feeding an iron-restricted diet, training for and conducting phlebotomies when possible, and impregnating females to mitigate iron loads. We hope this letter stimulates continued discussion and research in the zoo community, as we still have much to learn about rhinos and have shared goals of maximizing animal health and ensuring species survival.

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LITERATURE CITED

1. Bacon BR, Adams P, Kowdley KV, Powell LW, Tavill AS. Diagnosis and management of hemochromatosis: 2011 practice guideline by the American Association for the Study of Liver Diseases. Hepatology. 2011;54:328–343.
2. Bassett ML, Halliday JW, Ferris RA, Powell LW. Diagnosis of hemochromatosis in young subjects: predictive accuracy of biochemical screening tests. Gastroenterology. 1984;87:628–633.
3. Beutler E, Hoffbrand AV, Cool JD. Iron deficiency and overload. Hematology. 2003:40–61.
4. Boser P, Mordashova Y, Maasland M, Trommer I, Lorenz H, Hafner M, Seemann D, Mueller BK, Popp A. Quantification of hepcidin-related iron accumulation in the rat liver. Toxicol Pathol. 2016;44:259–266.
5. Bothwell TH. Overview and mechanisms of iron regulation. Nutr Rev. 1995;53:237–245.
6. Deugnier Y, Turlin B. Pathology of hepatic iron overload. World J Gastroenterol. 2007;13:4755–4760.
7. Dierenfeld ES, Kilbourn A, Karesh W, Bosi E, Andau M, Alsisto S. Intake, utilization and composition of browses consumed by the Sumatran rhinoceros (*Dicerorhinus sumatrensis harissoni*) in captivity in Sabah, Malaysia. Zoo Biol. 2006;25:417–431.
8. Finch CA, Stray S, Huebers HA, Bellotti V, Lipschitz DA, Cook JD, Pippard MJ. Plasma ferritin determination as a diagnostic tool. West J Med. 1986;145:657–663.
9. Kanwar P, Kowdley KV. Diagnosis and treatment of hereditary hemochromatosis: an update. Expert Rev Gastroenterol Hepatol. 2013;7:517–530.
10. Kock N, Foggin C, Kock MD, Kock R. Hemosiderosis in the black rhinoceros (*Diceros bicornis*): a comparison of free-ranging and recently captured with translocated and captive animals. J Zoo Wildl Med. 1992;23:230–234.
11. Kohgo Y, Ikuta K, Ohtake T, Torimoto Y, Kato J. Body iron metabolism and pathophysiology of iron overload. Int J Hematol. 2008;88:7–15.
12. Olias P, Mundhenk L, Bothe M, Ochs A, Gruber AD, Klopfleisch R. Iron overload syndrome in the black rhinoceros (*Diceros bicornis*): microscopical lesions and comparison with other rhinoceros species. J Comp Pathol. 2012;147:542–549.
13. Olivieri NF, Brittenham GM. Iron-chelating therapy and the treatment of thalassemia. Blood. 1997;89:739–761.
14. Paglia DE, Tsu I-H. Review of laboratory and necropsy evidence for iron storage disease acquired by browser rhinoceroses. J Zoo Wildl Med. 2012;43:S92–S104.
15. Powell LW, Seckington RC, Deugnier Y. Haemochromatosis. Lancet. 2016;388:706–716.
16. Roth TL, Rinhart PR, Kroll JL. Serum ferritin concentration is not a reliable biomarker of iron overload disorder progression or hemochromatosis in the Sumatran rhinoceros. J Zoo Wildl. Med. 2017;48:645–658.
17. Schook MW, Wildt DE, Raghanti MA, Wolfe BA, Dennis PM. Increased inflammation and decreased insulin sensitivity indicate metabolic disturbances in zoo-managed compared to free-ranging black rhinoceros (*Diceros bicornis*). Gen Comp Endocrinol. 2015;217–218:10–19.
18. Smith JE, Moore K, Cipriano JE, Morris PG. Serum ferritin as a measure of stored iron in horses. J Nutr. 1984;114:677–681.
19. St. Pierre TGS, Clark PR, Chua-anusorn W, Fleming AJ, Jeffrey GP, Olynyk JK, Pootrakul P, Robins E, Lindeman R. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. Blood. 2005;105:855–861.
20. Stasiak, IM, Smith DA, Ganz T, Crawshaw GJ, Hammermueller JD, Bienzle D, Lillie BN. Iron storage disease (hemochromatosis) and hepcidin response to iron load in two species of pteropodid fruit bats relative to the common vampire bat. J Comp Physiol B. 2018 [Internet]. Available from https://doi.org/10.1007/s00360-018-1155-4
21. Wang W, Knovich MA, Coffman LG, Torti FM, Torti SV. Serum ferritin: past, present and future. Biochim Biophys Acta. 2010;1800:760–769.

AUTHOR'S REPLY

Dear Editor:

This letter is written in response to the letter to the editor regarding the manuscript entitled "Serum ferritin concentration is not a reliable biomarker of iron overload disorder (IOD) progression or hemochromatosis in the Sumatran rhinoceros (*Dicerorhinus sumatrensis*)." Whereas I appreciate the letter authors' attempts to argue for the value of our lab's rhino ferritin-specific assay, I disagree with their suggestion that the data "highlight the utility of the assay as part of prevention and treatment of IOD." As detailed in the article, our study revealed evidence that serum ferritin concentration is not a reliable biomarker of IOD progression or hemochromatosis in the Sumatran rhinoceros. Below, I have tried to summarize the rationale behind our conclusions succinctly.

The underlying premise for this research is that serum ferritin concentrations increase with escalating body iron load. Our conclusions are based on logical arguments regarding the data's failure to support that premise, which include the following points:

1. The highest serum ferritin concentrations should have been associated with the rhino that had the highest liver iron concentrations; however, the rhino with the highest mean ferritin contained the lowest liver iron.
2. Serum ferritin values should have been higher in rhinos that developed hemochromatosis than in rhinos that did not develop hemochromatosis; the

rhino with the highest mean serum ferritin concentration did not develop hemochromatosis and displayed only mild hemosiderosis postmortem, whereas mean serum ferritin of one female that died of hemochromatosis was not comparatively high.

3. If reflective of disease progression, serum ferritin profiles should be similar among rhinos developing hemochromatosis; the two rhinos that died of the disease had very dissimilar profiles.
4. Natural liver iron accumulation occurs slowly over an extended period. Therefore, even if pregnancy was responsible for the fluctuating profile in the one animal, the data provide further evidence that ferritin is not a reliable indicator of IOD progression because ferritin was at baseline levels throughout much of this female's life, during which she must have been accumulating liver iron. These data directly contradict the letter authors' statement that "[T]hese animals had increasing serum ferritin over time." Mean serum ferritin concentration during the last 3 yr of this rhino's life prior to symptoms (January 2006–January 2009; 1,410 ng/ml) was lower than that for the 3 yr prior (January 2003–December 2006; 1,922 ng/ml), and neither mean would be considered high when compared to mean values for rhinos that did not develop hemochromatosis (1,267–2,604 ng/ml).
5. Serum ferritin concentrations should have been significantly elevated the year prior to clinical symptoms associated with iron-induced liver failure. Yet, in one of the two animals that died of hemochromatosis, serum ferritin concentrations in samples collected during the 12-mo, nonpregnant interval prior to symptoms were 1,143, 2,001, and 1,545 ng/ml. These values are not different from those measured in rhinos that did not develop hemochromatosis, and not much higher than that measured in a sample from a recently wild-caught Sumatran rhino (1,117 ng/ml).

Considering that frequent large-volume phlebotomy, a tremendous undertaking for staff and animals alike, currently is the only treatment available for rhinos suffering from excessive iron storage, there is a need for a diagnostic test that can accurately distinguish those rhinos with markedly elevated iron stores (moderate to severe hemosiderosis) that are likely to develop hemochromatosis versus those that have some excess, but non–life-threatening, iron stores (mild hemosiderosis), and are unlikely to experience iron-related organ damage. Based on the variable and inconsistent serum ferritin concentrations measured in this study, if I were a clinician (which admittedly, I am not), I would not diagnose a rhino's IOD condition and recommend intervention (or not) based on serum ferritin concentrations. The odds of false-positive and even false-negative diagnoses are too great. Therefore, I thought it prudent to share these data with the veterinary community. Although this study contained a small sample size, a follow-up, larger study by our lab in black rhinos[1] provides even stronger evidence supporting these initial conclusions regarding the unreliability of serum ferritin as a biomarker of IOD progression in the rhinoceros. To be clear, we are not arguing that these rhinos are not storing some iron in the form of hemosiderosis, only that serum ferritin does not accurately reflect IOD progression in this taxon. Of course, every clinician can and will decide for themselves how these results impact decisions they make regarding the health care of their rhinos. It is simply our responsibility to share our scientific findings with the community so that informed decisions can be made.

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LITERATURE CITED

1. Wojtusik J, Roth TL. Investigation of factors potentially associated with serum ferritin concentrations in the black rhinoceros (*Diceros bicornis*) using a validated rhinoceros specific assay. J Zoo Wildl Med. 2018;49(2):297–306.