(TfR-2), was identified that had G28A amino acid substitution. It remains to be determined if this substitution alters TfR-2 function. We next focused on differences potentially affecting red blood cell (RBC) development and survival, since such defects also cause ISD in humans and mice. Using unbiased mRNA sequencing of liver and spleen mRNA, we identified two mutations in proteins important for RBC function. These mutations were unique to black rhinoceroses compared to white rhinos and all other mammalian species. One mutation was identified in the SLC28a2 gene, which codes for the concentrative transporter for adenosine.[4] Since adenosine salvage is essential for maintenance of BC adenine nucleotides,[6] this mutation may be relevant to the extremely low (2-5%) RBC reserves of adenosine triphosphate (ATP) that are uniquely characteristic of rhinoceroses compared to almost all other mammals. Another mutation was found in EPB4.1, an erythrocyte membrane protein. Functional consequences of these mutations remain to be determined, but they could enhance RBC vulnerability to environmental stresses mediated by ROS, initiating a cycle of low-grade hemolysis and inducing increased iron absorption. Iron-catalyzed ROS in turn could worsen RBC damage, as has been observed in β-thalassemia, thus causing a vicious cycle of RBC destruction and iron overload. Understanding the genetic basis of RBC vulnerability and predisposition to ISD in black rhinoceroses should contribute to more effective captive management of browser rhinos, tapirs and other ISD-susceptible species.

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Case report: treatment of iron storage disease in a black rhinoceros (*Diceros bicornis*)in western Europe

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In January 2015 Vungu, a 15 year-old black rhino male, housed at the Rotterdam Zoo since 2013, became less active, had less appetite and laid down for prolonged periods. Weekly blood samples were collected for hematology and clinical chemistry. As black rhinos are sensitive for iron storage disease, a monitoring program for this disease was initiated[1,2]. Monthly serum samples were sent to the Veterinary Faculty in Utrecht for measuring serum iron (Fe2+), iron saturation and total iron binding capacity. GGT levels were very high (up to 131U/L, ref: 6-54U/L3) and iron saturation was up to 99%. In most species a saturation of maximum 70% is acceptable. In horses GGT is a reliable indicator for liver damage. Attempts to visualize the liver by ultrasonographic examination failed. Because of the concerns for his physical condition and behavior, a long-term large volume phlebotomy treatment was initiated. At first the procedure was done under general anesthesia (a combination of Immobilon (2.25mg

etorphine/ml, Pharmacy Veterinary Faculty Utrecht) with Zalopine®(10mg medetomidine/ml, Orion Pharma Animal Health). After 1 phlebotomy under general anesthesia (volume 8,5L) and an intensive successful training program for conditioned blood collection, phlebotomy could be performed without sedation. After 13 months and a 22 phlebotomies (volume varied between 0,2-6,8L) serum GGT decreased to 66U/Land the iron saturation dropped to below 80%. His general condition improved dramatically, including his appetite and activity and even hairs on the tip of his ears have grown back.

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Validation of a rhino specific ferritin enzyme immunoassay for the black rhinoceros (*Diceros bicornis*) for assessing the impact of individual and environmental factors on serum ferritin concentrations.

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Hemochromatosis (iron overload disease) is a potentially fatal disease that sometimes affects the critically endangered black rhinoceros (*Diceros bicornis*). Therefore, it is important to develop methods for monitoring the progression of iron storage (hemosiderosis), diagnosing the disease state, evaluating treatment efficacy, and assessing environmental factors that may impact iron absorption in this species. Ferritin is an acute phase protein complex that binds iron for storage and is used to measure total iron stores. Traditionally, an enzyme immunoassay (EIA) developed for horses was used to measure rhino ferritin. However, ferritin is species-specific, and recent inconsistencies in the equine EIA results prompted our lab to create a rhino-specific assay originally developed and validated for Sumatran rhinoceros (*Dicerorhinus sumatrensis*) ferritin. Our goal was to validate this assay for the black rhino and to investigate the influence of several environmental and individual factors on ferritin concentrations. Ferritin was isolated from black rhino liver then subjected to gel electrophoresis, using equine and Sumatran rhino ferritin as positive controls to verify sample purity. Staining with potassium ferricyanide confirmed the isolated protein was positive for iron. Western blot analysis and a parallelism were used to validate cross-reactivity of the Sumatran rhino ferritin antibody with black rhino ferritin. Serum samples (n= 681) collected during 1990-2016 from 28 black rhinos ranging in age from <1 to 32 years and maintained at 11 U.S. institutions were analyzed using the rhino ferritin EIA. Data were analyzed by NOVA and correlation analysis with the following factors included: individual, subspecies, location, sex, age, age group, collection date (month, year, season), captive/wild status of individual at birth and of parents, and time in captivity. All data are reported as means± SEM. The mean serum ferritin concentration for all samples was 3090.5 ± 98.4 ng/ml (range: 84.6 to 19,296.1 ng/ml). Concentrations differed among individuals ($P < 0.05$) and were higher ($P < 0.05$) in males (3539.2± 108.6 ng/ml) than females (1094.7 ± 124.0). Ferritin was lower ($P<0.05$)in pre-pubertal (< 5 years old) individuals (1761.8 ± 152.7 ng/ml) than adults(6-25 years; 3392.7 ± 118.3) and seniors (26+ years; 3570.7 ± 347.3). Ferritin levels in adults and seniors were not different ($P> 0.05$), but the senior sample size was small limiting the possibility of