• cement coverings of walls,

• feeding elephants outside the enclosure, forcing them to stretch with their trunks and bump their tusks on the enclosure boundary,

• chaining elephants,

• using cement flooring (especially if smooth). This to facilitates rapid cleaning of pens, but the smooth wet surface increases the risk of slipping,

Good management practices include keeping tusk trimming to a minimum, understanding pulp anatomy in order to prevent exposure when trimming, and managing herd structure correctly. When it comes to successful treatment, the identification of pulp exposure when tusks are fractured/cut is paramount. Immediate treatment of the pulp (partial pulpectomy) with appropriate medications and the effective sealing of the tusk has been shown to have excellent results. Prolonged periods with no treatment are not recommended as this leads to destruction of pulp tissue and ultimately pulp volume. Tusks with decreased pulp volume may slow down completely with respect to tusk growth (new ivory formation), or may continue to be infected - ultimately leading to pulp necrosis necessitating tusk extraction. In addition, abnormal ivory formation has been described in elephants, with chronic infection being one of the causal mechanisms. This abnormal ivory can complicate the extraction process even further.

## Development of a rhino ferritin specific assay for determining the association between serum ferritin concentrations and hemochromatosis in the Sumatran rhinoceros (Dicerorhinus sumatrensis)

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Among the challenges associated with iron overload disorder in rhinos is the inability to distinguish individuals developing and/or suffering from hemochromatosis (iron overload disease) from those merely experiencing hemosiderosis (iron deposits in organ tissues without associated organ damage). Although neither condition is desirable, the former can be fatal, whereas the latter may be tolerated throughout a long, productive life. Serum ferritin concentration is considered the best measure of total body iron stores in many species, but ferritin is species specific and an acute phase protein that increases in response to many inflammatory reactions. When Sumatran rhino serum ferritin concentrations were measured using an antibody for horse ferritin, values for an individual that died of hemochromatosis were no different during the eight months she exhibited clinical signs of disease than values for an old Sumatran rhino that died many years later of other causes (range; 740-2,650 and 382–2,445 ng/ml, respectively). The aim of this study was to develop an enzyme immunoassay (EIA) using Sumatran rhino ferritin specific antibodies to definitively determine if ferritin is a reliable biomarker of hemochromatosis in this species. Through a multi-step process, ferritin was isolated from Sumatran rhino liver tissue. Sample purity was confirmed via polyacrylamide gel electrophoresis and Coomassie blue staining using purified horse ferritin as a control. Staining was also performed with potassium ferricyanide to confirm the presence of iron in the protein bands. Several monoclonal mouse antibodies were generated against the isolated protein at the University of Georgia Bioexpression & Fermentation Facility. Two top-performing monoclonal IgG antibodies were validated for their specificity to rhino ferritin by Western blot analysis and were used to develop a sandwich EIA. Serum samples banked during the lifetimes of five Sumatran rhinos maintained in U.S. Zoos and spanning 4 to 15 years for each individual were retrospectively analyzed for ferritin concentrations. One rhino had produced three calves prior to her death from hemochromatosis, another died of hemochromatosis without reproducing, two had lived long lives and died of other causes and one was still alive. In addition, single serum samples were analyzed from each of nine Sumatran rhinos maintained in S.E. Asia, none of which died of hemochromatosis. For U.S. housed rhinos, serum ferritin

concentrations ranged from 348-7,525 ng/ml across all samples and all rhinos with individual overall means ranging from  $1,267 \pm 227$  to  $2,603 \pm 1,200$  ng/ml. For rhinos maintained in S.E. Asia, the mean serum ferritin concentration was  $(4,985 \pm 4,197 \text{ ng/ml})$  with individual values ranging from 858 to 14,900 ng/ml. Results from the horse and rhino assays differed with correlation coefficients ranging from negative to highly positive values (R = -.36 to .93) for individual profiles. Data from this study indicate that Sumatran rhino serum ferritin concentrations: 1) are not accurately determined with antibodies to horse ferritin; 2) are dynamic over time; 3) may decrease during pregnancy; 4) are elevated in rhinos suffering from hemochromatosis; and 5) are elevated in many rhinos not suffering from hemochromatosis. In conclusion, serum ferritin concentration is not diagnostic of hemochromatosis in the Sumatran rhino. (This study was funded by a grant from Mr. and Mrs. Jeremy S. Hilton and Family with supplemental support provided by Dr. Tom and Rita Bell.)

## Clinical case: Gross examination on left rear leg of Sumatran rhinoceros (*Dicerorinus sumatrensis*) Harapan by using digital X-ray

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The Sumatran rhinoceros (Dicerorhinus sumatrensis) is one of the most critically endangered of large mammals on earth today. Based on IUCN's Red List of Threated Species, Sumatran rhinoceros is classified as Critically Endangered (and also listed in CITES Appendix 1). The critically endangered Sumatran rhino's future is questionable with a shrinking wild population currently estimated at fewer than 100 rhinos and which needs serious action to save this species. Sumatran Rhino Sanctuary (SRS) is a breeding center for Sumatran rhinoceros in its native habitat run by Yayasan Badak Indonesia (YABI) in collaboration with the Ministry for Environment and Forestry, Government of Indonesia, and financially supported by the International Rhino Foundation (IRF). This sanctuary of about 100 ha is located in Way Kambas National Park. Since 1985, about forty Sumatran rhinoceros have been captured from the wild and translocated around the world. Three wild caught Sumatran rhinoceros were relocated to the Cincinnati Zoo and they successfully produced three calves - Andalas, Suci, and Harapan. Andalas and Harapan were returned to Indonesia, but Suci passed away due to health problems. Harapan, an 8 year old male, was moved to Indonesia by using air, land, and sea transportation. The journey took about 60 hours and he was placed in a crate about 3m x 1.5m x 2 m (I x w x h). A few days after arrival, he was observed with a limp on his left rear leg. Based on daily observation, Harapan's problem was thought to be located in the bone or tendon in the digitalis pedis area. X-ray examination was done without sedation with the rhino inside the stall. The crack observed on X-ray healed by itself, but it took 3-4 months due to the minimal blood vascularization of the phalanx bone. The bone is also actively used when the animal walked. Oral medicines like NSAIDs were not recommended for this case due to long term use. Symptomatic treatment was provided by giving calcium supplement (bone supplement) and by putting mattresses inside his stall for cushioning.