DEVELOPMENT OF FIELD ASSAY FOR EVALUATION OF WHITE RHINOCEROS NEUTROPHIL FUNCTION AS A STRESS MARKER

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Abstract

Kruger National Park (KNP) is the primary source of translocated white rhinoceroses (*Ceratotherium simum*) in South Africa. Capture and transport of the rhinoceroses is a highly successful procedure. However, some relocated rhinoceroses present with fertility problems in the 1–2 years post capture. Novel research has shown that one can assess stress using respiratory neutrophil burst levels in several species (Huber *et al.* 2006 Protoplasma **229**, 221–224; Weyts *et al.* 1998 Dev. Comp. Immunol. **22**, 563–572). The hypothesis is that some rhinoceroses respond poorly to stress. The response to stress might be measurable by a neutrophil function (NF) assay, and NF depression might identify adversely stressed animals. The aim was to develop an in-the-bush test for NF and evaluate the changes in neutrophil activity during capture and loading into transportation crates. Neutrophil function was assayed using a portable luminometer (3M Clean-Trace™, 3M, St. Paul, MN, USA) while driving from one capture to the next. For the reaction mixture, 500 μL of PBS was incubated with 10 μL of fresh heparinized blood at 37.6°C. The incubator unit was power by a 230 V convertor in the vehicle. After 5 min of incubation, 100 μL of luminol was added. This sample was read in the luminometer as a blank and then to the sample, 100 μL of 12-O-tetradecanoylphorbol-13-acetate (TPA), also commonly known as phorbol 12-myristate 13-acetate (PMA), a diester of phorbol, was added. The luminescence readings or relative light units (RLU) were read at 2.5 min and then 5 min after PMA addition. All samples were prepared in triplicate. The readings were then taken every 5 min for 65 min. Two samples of blood were evaluated for each rhinoceros: the first sample at capture (anaesthetic induction) and then a second sample after loading of the rhinoceros into the transport crate, 20 to 30 min later. Two response curves were produced for each rhinoceros using the means of the triplicate readings. The curves were then transformed with a trend line and the area under the curve (AUC) was calculated. The 2 AUC for rhinoceros were then compared statistically using Wilcoxon rank regression, with $P < 0.05$ considered statistically different. Three distinct response curve patters were seen. Sixty-two percent (16/26) of the rhinoceroses had no statistical ($P < 0.05$) difference between capture and loading samples. In 5 of 26 (19%), the loading AUC was statistically greater than the capture sample, and in 5 of 26 (19%), the capture AUC was statistically greater than the loading sample. In conclusion, NF can be assayed in the field using the blood of wild caught rhinoceroses. The assay is repeatable and can distinguish 3 populations of wild caught rhinoceroses. The hope is that future research will allow us to identify rhinoceroses that have negative stress reactions and to change the capture conditions to make the process less stressful.

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