Comparison of haematology and specific serum biochemistry variables between anaesthetised non-injured and injured southern white rhinoceros (*Ceratotherium simum simum*)

by

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Dissertation submitted in partial fulfilment of the requirements for the degree

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Declaration of Originality

I, Jacques Pieter du Preez, hereby declare that the research presented in this dissertation was conceived and executed by myself under the guidance of my supervisor and co-supervisors.

Neither the substance nor any part of this dissertation has been submitted in the past, or is to be submitted for a degree at the University of Pretoria or any other tertiary institution, apart from for the purposes of the degree I am currently enrolled for.

This dissertation is presented in partial fulfilment of the requirements for the degree Master of Science (MSc) in Veterinary Science under the Department of Companion Animal Clinical Studies of the Faculty of Veterinary Science of the University of Pretoria.

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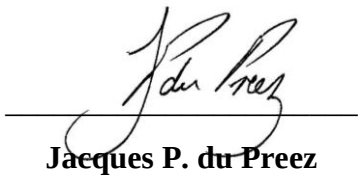

Jacques P. du Preez

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Animal Ethics Committee Approval Certificate

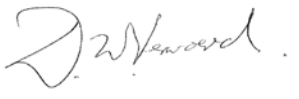
PROJECT TITLE	**Comparing haematological and biochemical parameters of healthy and critically injured rhinoceroses to determine prognostic indicators for survivability**
PROJECT NUMBER	**V033-14**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. JP du Preez**

STUDENT NUMBER (where applicable)	**27017932**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	**White Rhinoceros** ***(Ceratotherium simum simum)***	**Black Rhinoceros** ***(Diceros bicornis bicornis)***
NUMBER OF ANIMALS	**100 in total**	
Approval period to use animals for research/testing purposes		**June 2014 – May 2015**
SUPERVISOR	**Prof. G Steenkamp**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 26 May 2014
CHAIRMAN: UP Animal Ethics Committee	Signature 

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Too many people contributed to this project to list here, and in fear of leaving anyone out, I would like to thank each and every person and organisation who played a role in making this project and this dissertation a reality. I hereby also extend my sincere appreciation and thanks to the following people and organisations by name, without whom this project would not have been possible:

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- The Wilderness Foundation and the SeaWorld and Busch Gardens Conservation Fund for their generous donation of funding towards this project, without which none of this would have been possible.

WILDERNESS FOUNDATION
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RHINO PROTECTION INITIATIVE

Dedication

Figure A-1: Poaching Survivor from Eastern Cape, South Africa, called Hope. (Photo credit: Adrian Stern and Saving the Survivors)

To Hope and all the other poaching victims, both survivors and non-survivors, of her species, who inspired this research project and allowed me to learn from them and to share that knowledge with the world.

In Memory of:

My father, Willie du Preez (1955/09/02 – 2018/02/07), whose love for nature and animals in particular inspired my career and whose motivation and support to follow my dreams will forever mean the world to me. Words cannot express the appreciation I have for all you have done for me through the years. Thank you! This is for you, Dad!

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List of Abbreviations and Definitions

>	Greater than
<	Less than
±	Plus and minus
^{o}C	Degrees Celsius
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BUN	Blood urea nitrogen
Ca	Total calcium
Ca^{++}	Ionised calcium
CI	Confidence Interval
CITES	Convention on International Trade in Endangered Species
Cl^{-}	Chloride
CK	Creatine kinase
Cr	Creatinine
DEA	Department of Environmental Affairs, South Africa
Dr.	Doctor
EDTA	Ethylenediaminetetraacetic acid
e.g.	for example
et al.	and others
fL	femtolitre
g	G-force
G	gauge as in needle gauge
GGT	Gamma Glutamyl transferase
GI	Gastrointestinal
GLDH	Glutamate dehydrogenase
ISIS	International Species Information System
K^{+}	Potassium
KNP	Kruger National Park
Max.	Maximum
Measurand	quantity intended to be measured (1)
Mg^{2+}	Magnesium

Min.	Minimum
mmol	millimole
µmol	micromole
Na^+	Sodium
OVAH	Onderstepoort Veterinary Academic Hospital
pg	pico-gram
Prof.	Professor
Rhino	Contraction of the word “rhinoceros”
RI	Reference Interval
SD	Standard Deviation
SEM	Standard error of the mean
SIP	Serum Inorganic Phosphate
STS	Saving the Survivors
ZIMS	Zoological Information Management System

Chapter 1: Introduction

1.1 General Literature Review:

The rhino poaching issue in South Africa is arguably one of the, if not the most important environmental crime crisis the country has ever faced. As custodians of more than 75% of the world's remaining rhinos, a growing responsibility rests on South Africans to find a solution for this poaching problem. The annual statistics for South Africa show an alarming escalation in the numbers of rhinos poached in recent years. From 1990 to 2005, the average annual poaching numbers were only 14 per year. This number increased to 83 in 2008, and disturbingly peaked at 1215 rhinos poached in 2014 (2). During 2015 the number slightly decreased to 1175 (3) while the official numbers for 2016 and 2017 were 1054 and 1028 respectively (4). According to the most recent media release from the Department of Environmental Affairs (DEA), 508 rhinos were poached by 31 August 2018 (5). These numbers show a small yet steady decline in poaching numbers although the total number still remains alarmingly high. The vast majority of rhinos being poached are the southern white rhino subspecies (*Ceratotherium simum simum*).

The South African government has a multi-disciplinary approach in its efforts to fight rhino poaching. The main areas of focus are law enforcement (better policing and monitoring of high risk areas, more arrests and prosecutions, tougher sentences upon conviction, better border control and the adoption of various technological advances) and population management (which includes translocations and support for the care of orphaned calves) (4).

The situation in the Kruger National Park (KNP), home to the world's largest remaining rhino population, is deteriorating alarmingly. The number of animals poached in KNP as a percentage of national figures has increased from 43% in 2010 (146 out of 333 poached) to 70% in 2015 (826 out of 1175 poached) (3). For 2017, this percentage was 49% (504 out of 1028 poached), suggesting a possible shift in the poaching dynamics in South Africa (4). Ferreira *et al.* (6) modelled the number of animals poached in 2012 (668 rhinos poached in 2012 (2)) to show that at that rate of poaching increase, the numbers poached would exceed the recruitment from births by 2016. This estimated number was already surpassed in 2013 (1004 rhinos poached in 2013 (2)), indicating the intensification of poaching despite all the

above mentioned efforts to curb it. They also predicted that at poaching rates in 2012, rhinos are likely to become extinct in 2025 (6). This might even become a reality sooner if the current rate of poaching, as seen to date in 2018, is to continue.

Substantial effort is being placed on trying to curb the illegal trade in wildlife products in Southern Africa without any indication that a reduction in the rate of poaching will occur in the foreseeable future. It is widely recognised that no single solution exists to solve the poaching crisis. While local protection efforts remain at the heart of government directed interventions, global awareness, education and reduction in demand are vital, shared responsibilities which extend across all social and economic interest groups and hold potential implications of both success and failure for all species affected by the illegal wildlife trade globally.

In the first conference on illegal wildlife trade in London in 2014, fifty countries and international organisations committed to various measures in an effort to help curb illegal trade and to protect endangered species (7). These measures include improvement and creation of legislation, where needed, to treat wildlife trafficking and poaching as serious crimes, reduction in the demand for these wildlife products and creating and supporting alternative livelihoods for communities affected by poaching and trafficking (7). Another important step was governments renouncing the use of products from species threatened by extinction (7). The United Kingdom committed itself to support anti-poaching and wildlife trafficking efforts globally (7). In the subsequent two conferences in Kasane, Botswana in 2015 and in Hanoi, Vietnam in 2016, the implementation of these efforts were further established and efforts were evaluated. In 2018, London again hosted the 4th conference on illegal wildlife trade to review progress of proposed government actions and reaffirm commitments through implementing further initiatives. Following the Hanoi conference in 2016, the governments and organisations involved, committed to:

- strengthening engagement with the private sector and online retailers to put in place measures to prevent the sale of illegal wildlife products;
- renouncing the use of products from species threatened with extinction;
- supporting governments and financial institutions to detect, investigate, and disrupt money laundering and other financial crimes related to the illegal wildlife trade;

- strengthening cross-border co-ordination and support for regional wildlife law enforcement networks;
- engaging communities living with wildlife as active partners in conservation, through reducing human-wildlife conflict and supporting community efforts to advance their rights and capacity to manage and benefit from wildlife and their habitats;
- further analysis to better understand the links between wildlife crime and other organized crimes, and to explore links to terrorism (8).

In South Africa, following a Constitutional Court order in April 2017 upholding a High Court ruling in 2015, the moratorium banning domestic trade in rhino horn since 2009 was lifted (9). Despite the ruling, the last CITES meeting in Johannesburg (2016) upheld the international ban in the trade of rhino horn (9, 10).

In the background to all of these important efforts is the plight of individual animals that are wounded during poaching attempts. Wounded rhinos still remain a welfare issue and the management considerations and clinical interventions required to address the needs of these animals are poorly understood. The treatment of domestic animals for a variety of emergency situations has developed to advanced levels, but not to the same extent in wildlife, and not in free-living rhinos. To further this effort, and apply it to clinical cases, thereby enhancing the success of treating wounded rhinos, additional research is needed. The research results presented in this dissertation continued the process of understanding the injured rhino by answering some questions but at the same time enabling future researchers to ask more specific and pertinent questions.

1.1.1 Problems associated with treating injured wild rhinos

Detailed assessments of health and treatment of wild, free-ranging rhinos in their habitat requires restraint in the form of immobilisation (11). Etorphine combinations delivered remotely by dart are routinely used for immobilization (12). The multitude of physiological alterations during immobilisation in healthy animals such as respiratory depression, hypercapnia, hypoxia, hypertension, pulmonary shunting and V/Q mismatching are well documented in white, black and Indian rhinos as well as the African elephant and bighorn sheep (13-19). It is therefore essential to keep repeated immobilisations in the severely compromised animal to an absolute minimum in both duration and frequency. Often injured rhinos are found with severe facial wounds where the horns and the rostral parts of the face

are removed. These wounds need to be covered to promote wound healing and the dressing replaced at least once every four to six weeks. Ideally, such patients need to be kept in a small camp or boma for this purpose. It is a frequently observed phenomenon that adult wild white rhinos that are confined to a boma do not adjust to captivity well and often become anorexic (20). A recent study however demonstrated that most white rhinos took to being fed lucerne or other fodder within 14 to 21 days in a captive situation (21). Despite this, these severely injured animals are often already compromised physiologically and any period of anorexia is not ideal, putting further constraints on the treatment options available to the clinician.

1.1.2 Biological data

Baseline haematological and serum biochemical values have been evaluated for 'healthy' captive (22) and free-living (11) white rhinos. The Species360 database, previously known as the International Species Information System (ISIS), managed by ZIMS, also has baseline clinical pathological measurands in their system (23). Baseline serum biochemical values for the white rhino have been published (11, 24), and a recent study evaluating selective serum and plasma biochemical measurands in white rhinos in the Kruger National Park (KNP) has been published (25). In another recent study on white rhinos from the KNP, baseline haematological measurands were also evaluated. (26). Haematological values for black rhinos have also been reported (27). Baseline biological data (including haematology and serum biochemistry) have been collected and reported by Kock and du Toit from black rhino in Zimbabwe (28), and black rhino haematological and biochemical values have been published for a South African population (29). Data on physiological measurands exists for the unrestrained white rhinos (30) and for rhinos under immobilisation, which produces significant physiological alterations (14, 31). Following the method comparison between a point of care and wet chemistry analyser, reference intervals for selected plasma biochemistry measurands have been determined for white rhinos (32). The effects of translocation on haematological and biochemical measurands of the black rhino have also been documented (16, 33). Haematological and serum biochemical values have been published for the Northern white rhino subspecies (29, 34), however no published reports of similar studies in the Javan and Indian rhinos could be found. A study in 2013 looked at the haematology and serum biochemistry of five Sumatran rhinos (35). A handheld lactate meter was validated for field use in the white rhino (36). Despite all the research done on determining baseline values of different rhino populations, no studies have described the alterations of physiological and clinical laboratory values that occur in injured rhinos.

On closer examination of the many haematology and biochemistry studies performed, various inconsistencies in the methods of sample collection and sample handling, before laboratory analysis, are evident. In some instances the exact method of analysis is not described, and in others, the methods differ between studies. Inadequate sample sizes were often a limitation in most of these studies. These factors bring into question the accuracy and reliability of these "normal" values when determining prognosis in an injured rhino. There is therefore still a need to collect more samples from healthy rhinos and analyse them in a controlled and standardised way to obtain accurate measurands to establish reliable "normal" values.

In other species, the literature (37-39) is not conclusive regarding the stability of all measurands included in this study in long term freezer storage at both -20^{o}C and -80^{o}C. Because the stability of measurands, especially in white rhino, is not sufficiently known, the use of samples, especially those greater than one year old (40), to determine reference intervals should be done with caution.

1.1.3 Prognostic value of Clinical and Biological Measurands

In order to predict prognosis in clinical cases, clinicians would need to combine their clinical evaluation with a variety of results from various diagnostic and laboratory tests. Subsequently, accurate treatment is more likely, resulting in the better possible outcomes of cases. In human medicine, various scoring systems like the APACHE system exist (41, 42) to aid clinicians dealing with critical patients in predicting outcomes and make the relevant adjustment in treatment regimens. These systems take into account large sets of data including basic clinical examination findings and detailed laboratory test results to determine severity of disease in critical care human patients in an effort to provide prognosis. The classification of injuries, especially gunshot injuries, also has proved invaluable for prognostication (43). The same holistic approach of taking into account a large set of information to determine prognosis, is also seen in outcome prediction in other species, such as dogs, horses and in one study of Bighorn sheep. (19, 44-46). The current study aims to do the same for white rhinos as no such studies exist for these animals.

There is a dearth of information regarding critically injured rhinos surviving poaching events. The numbers of animals that initially survive a poaching event are not known, nor the numbers that survive long term. Furthermore, the injuries sustained by these rhinos have not

been described or categorized in detail, and no data exists on the veterinary care and outcomes of injured rhinos that initially survive poaching events. A description of the numbers of animals involved, types of poaching methods employed, injuries sustained, results of diagnostic tests, treatments being used and final outcome, will enable the veterinary profession, rhino owners, wildlife rangers and all other interested parties to have a greater understanding of the problems faced and how to address them.

The lack of physiological and clinical laboratory data from injured rhinos limits effective treatment of injured animals and limits our ability to determine their prognosis. Neither are there any studies to evaluate clinical or laboratory measurands for use as indicators of prognosis in critically injured rhinos. Properly determining the physiological and clinical changes caused by injuries would form the basis for treatment and management of rhinos that are subject to similar poaching issues throughout their normal distribution range. These findings most likely would not only apply to white rhino, but also to other rhino species in Africa and Asia.

Prediction of survivability at the discovery of rhinos that have been shot or otherwise injured will be a useful management tool in terms of a reserve manager or veterinarian's response to a crisis.

Furthermore, identifying positive or negative prognostic indicators will enable a more evidence-based approach to prognostication in injured rhinos. More accurate prognostication will improve the welfare of injured rhinos in those cases where long-term survival is unlikely and euthanasia may be appropriate. Triage is the process of sorting patients into categories based on priority of treatment needed, in order to assign resources wisely (47). This is done taking into account various physical and physiological variables, including prognostic indicators (48). Establishing prognostic indicators for injured rhinos will enable more effective use of resources where time and money can be directed towards cases with a higher chance of success by aiding in the establishment of proper triage procedures. Identifying positive or negative prognostic indicators in rhinos may also help to refine treatment plans, and identify areas for future research.

1.2 Aims:

There were four main aims to this study:

1 Determine normal haematological and serum biochemistry reference intervals for healthy rhinos darted from the air with a standardised anaesthetic protocol.

2 Describe the clinical, haematological and serum chemistry findings in injured rhinos

3 Determine if there are any of the measurands that may aid in establishing a prognostication protocol for injured rhinos.

4 Establish prognostic indicators and develop protocols to determine prognosis for injured rhinos.

1.3 Objectives:

The specific objectives of this study included the following:

a. To establish "normal" reference interval values for all possible useful haematological and biochemical measurands from blood samples in normal healthy rhinos. Normal being defined as rhinos that were immobilised at the time of collection of the samples, thus taking into account the effect of the immobilising drugs and the method of immobilisation (from a helicopter) on the physiology.

b. To compare clinical, haematology, and blood chemistry data of samples from chemically immobilised injured rhinos that are still alive to those of chemically immobilised healthy individuals.

c. To investigate predictive values of survivability based on clinical, haematology and blood bio-chemistry evaluations done on injured rhinos.

The overall objective of this study is to allow for the development of criteria for the clinical evaluation of wounded rhinos, to help institute appropriate therapeutic interventions based on a thorough understanding of the pathophysiology caused by their injuries.

1.4 Hypotheses:

In this study the following hypotheses are tested:

1.4.1 H_0: Normal haematological and biochemical data collected in this study will not differ significantly from normal measurands already published and will thus confirm those values.

H_a: Normal haematological and biochemical data collected in this study will differ from previously determined and published normal values.

1.4.2 H_0: There will be no significant differences between specific haematology and serum biochemistry of chemically immobilised injured and healthy rhinos.

H_a: There will be significant differences between specific haematology and serum biochemistry of chemically immobilised injured and healthy rhinos.

Chapter 2: Materials and Methods

2.1 Study design and sample size

The study was an analytical prospective observational study and samples were collected over a 12 month period from May 2014 till May 2015. Our aim was to collect data from at least 50 rhinos in each of two groups in this study (see 2.3).

In cases where healthy rhinos were immobilised, one set of samples was collected at the time of immobilisation. The same set of samples was collected from those that were injured. However, in cases where some of these injured rhinos were seen again by a veterinarian for follow-up treatment, a subsequent sample set was taken at each visit in order to have serial sets of data during recovery.

Due to the questionable stability of stored samples, as discussed above under 1.1.2, it was decided that stored samples from the Mathebula *et al.* (25) study were too old for re-analysis as viable controls for this study.

2.2 Study setting

The project was based from the Onderstepoort Veterinary Academic Hospital (OVAH) of the Faculty of Veterinary Science on the Onderstepoort Campus of the University of Pretoria. All the blood samples collected for this study were analysed by the Clinical Pathology Laboratory at the OVAH.

2.3 Study animals

For the purpose of this study, the rhinos were divided into two groups:

Group 1: Non-injured rhinos to be used as control animals.

Group 2: Injured rhinos as a consequence of poaching or other traumatic events.

2.3.1 Group 1 (Non-Injured Rhinos):

Blood was collected from non-injured southern white rhinos from multiple locations throughout South Africa. Due to the fact that rhinos in this group were immobilised by different veterinarians in different locations, the immobilization drug and procedural

protocols differed slightly. However, all rhinos were immobilised with various doses combinations of etorphine (M99, Novartis, Kempton Park, South Africa, 1619), azaperone (Stresnil, Jansen Pharmaceutical Ltd., Halfway House, South Africa, 1685) and hyaluronidase (Hyalase, Kyron Laboratories, Benrose, South Africa, 2011). Drugs were administered intramuscularly using darts from various manufacturers, depending on the preference of the attending veterinarian. To stabilise the cardiorespiratory system, butorphanol was administered intravenously in an auricular vein within 10 minutes of immobilisation. These rhinos were immobilised for routine management purposes (mostly translocation and routine dehorning) and not specifically for the purposes of this project. Immobilisation was either from the ground or from a helicopter. The rhinos in this group were determined to be healthy based on a physical examination and body condition at the time of immobilisation.

2.3.2 Group 2 (Injured rhinos)

The injured population consisted of any injured southern white rhinos in South Africa that were attended to by a veterinarian after initially surviving a poaching event or other traumatising events. All rhinos were alive at the time of sampling. How long each animal survived after these events was recorded where possible. Attempts were also made to estimate, where exact time periods were not known, the time between the event and veterinary attendance by using a 4 point scoring system.

Table 2.1: Scoring system used to estimate time elapsed between poaching event and veterinary attendance.

SCORE:	**1**	**2**	**3**	**4**
Period:	Less than 8 hours	9-24 hours	25-96 hours	More than 97 hours

Figure 2-1: An example of the lower end of the spectrum of injured rhinos included in this study. The arrow indicates small calibre single gunshot wound. (Photo Credit: STS)

Figure 2-2: An example of the higher end of the spectrum of injuries included in this study. Here a severe facial wound involving both soft tissue and bone. (Photo Credit: STS)

2.3.3 Inclusion criteria

For Group 1, any non-injured white rhino that was in good condition and appeared clinically healthy and was immobilised for translocation or other management purposes was included in the study. Equally, for Group 2, all injured and traumatised white rhinos attended to by veterinarians anywhere in the country were considered for this study. Trauma to the rhinos included poaching events as well as interspecific aggression that caused injuries. A poaching event was classified as any unlawful attempt to dart, kill or injure a rhino in an effort to harvest its horn outside of normal park, reserve or veterinary management procedures where the removal of the horn is required. Horns in these animals may or may not have been removed.

The injured group was divided into survivors and non-survivors in order to examine the association between measurands and prognosis. A survivor was classified as any rhino that was alive when found, and survived for at least 21 days after the traumatic event. Where deaths occurred at a later stage in the survivors group, it was ruled due to causes unrelated to the traumatic event, such as complications related to husbandry. Non-survivors were rhinos that died or were euthanized for humane reasons within 21 days after initial veterinary attendance and sampling. The 21 day cut-off was decided upon based on the cases we have seen since all non-survivors died or were euthanized within this period in this study. Deaths in the non-survivor group were assumed to be directly or indirectly, as a result of complications from the traumatic event, due to the injuries sustained since no formal post mortem examinations were done.

2.3.4 Exclusion criteria

Any animal that was not healthy, meaning an animal that has some form of injury or was suffering from any detectable disease with clear clinical manifestations was excluded from the control group.

Conversely, the injured group did not include any animals that were healthy or had pathophysiology from any other illness other than trauma; only rhinos injured by some form of physical trauma were included in the study group.

2.4 Sampling methods

Immobilised healthy and injured rhinos

Blood samples were taken from immobilised rhinos into 4.0 ml BD Vacutainer® heparinised, EDTA, sodium fluoride & potassium oxalate, citrate and serum blood tubes (Becton, Dickinson and Company, Belliver Industrial Estate, Plymouth, PL6 7BP, UK) within the first 10 minutes after immobilisation. The tubes were filled to maximum vacuum capacity. A minimum of two serum and heparin tubes were collected, while one fluoride oxalate, EDTA and citrate tube were collected for each case. Blood was collected from the caudal auricular veins (back of the pinna) or the cephalic vein on the front limb in cases where the animals were in lateral recumbency, using a BD Vacutainer® One Use Holder (Becton, Dickinson and Company, Belliver Industrial Estate, Plymouth, PL6 7BP, UK) and a 21G BD Vacutainer® PrecisionGlide™ Multi-Sample Needle (Becton, Dickinson and Company, Belliver Industrial Estate, Plymouth, PL6 7BP, UK). After collection, samples were immediately placed in a cooler box with ice blocks until pre-analytical processing could be done at the closest available clinic or laboratory. The samples in the serum, heparin, fluoride oxalate and citrate tubes were centrifuged at 2000 g for 8 min and the plasma and serum decanted into 1ml aliquots (to facilitate future research) within a maximum of 6 hours after, but in most cases within 2 hours of sample collection. These aliquots were then kept refrigerated or on ice until they reached the laboratory at the OVAH for analysis. In cases where samples reached the lab after normal operating hours or could not reach the lab within 24 hours (samples from the KNP) these were kept frozen at -20 ^{o}C or -80 ^{o}C until the time of analysis. After analysis, all remaining plasma and serum were placed in the -80 ^{o}C freezer for long term storage at the OVAH. Blood in EDTA tubes was also transported to the lab at OVAH within 24 hours for complete blood count analysis.

Complete blood counts were performed on either the ADVIA® 2120i Hematology System (Siemens Healthcare GmbH, Henkestr. 127, 91052 Erlangen, Federal Republic of Germany) or the Cell-Dyn 3700CS Haematology analyser (Abbott Diagnostics, 5440 Patrick Henry Drive, Santa Clara, CA 95054, USA). The ADVIA Hematology System was used during normal working hours at the laboratory, while the Cell-Dyn analyser was used when samples arrived after hours.

Blood smears were made at the time of analysis from the blood in the EDTA tubes, according to standard OVAH laboratory protocol, and stained using Diff-Quick stain. These blood

smears were examined for differential counts and to screen for blood parasites. Parasites, where present, were only identified microscopically up to genus level.

All serum biochemistry, apart from cortisol, fibrinogen and ionised calcium, was performed using a wet chemistry analyser, Cobas® Integra 400 Plus from Roche (F. Hoffmann-La Roche Ltd., Grenzacherstrasse 124 4070 Basle, Switzerland). Cortisol was analysed using the Immulite 1000 analyser (Siemens Healthcare GmbH, Henkestr. 127, 91052 Erlangen, Federal Republic of Germany). Fibrinogen was analysed with the STart® 4 Haemostasis analyser (Diagnostica Stago SAS, Siège Social, 3 allée Thérésa, 92600 Asnières sur Seine, France) and ionised calcium was analysed using the Siemens Rapidpoint 348 or 405 (Siemens Healthcare GmbH, Henkestr. 127, 91052 Erlangen, Federal Republic of Germany). These two Rapidpoint analysers are simply two different models using equivalent methods of analysis with the same analytical principles.

2.5 Observations

Observational and clinical data were collected by myself in all the cases where I was involved with the injured animals and recorded on a data collection sheet developed for this purpose (Appendix A). Where it was not possible to attend to the injured rhinos, a data collection sheet was sent to the attending veterinarian (via email) and followed up with a telephone call. In certain cases, veterinarians alerted me beforehand that they will be attending to rhinos and requested the data collection sheet beforehand together with instructions on the samples needed. Where I was not present, samples were sent to the Clinical Pathology Laboratory in the OVAH by courier with same day or overnight delivery.

The data collected included:

1. Group 1:
    - Signalment: Species, age group (calf, sub-adult, adult), age (or estimated age), sex, weight (or estimated weight) in kg and habitus. Age was estimated subjectively by the attending veterinarian based on horn length (where present) and body size.
    - History: Reproductive status, previous disease or veterinary intervention, and date of previous immobilisation.

- Clinical examination: Heart rate (HR), respiratory rate (RR), peripheral pulse quality, mucous membrane colour and capillary refill time and temperature.
- Haematology: Red cell count (RCC), haemoglobin (Hb), haematocrit (HCT), white cell count (WCC), differential leukocyte count, mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC), red cell distribution width (RDW) and thrombocyte count.
- Serum biochemistry: Alkaline phosphatase (ALP), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), glutamate dehydrogenase (GLDH), total serum protein (TSP), albumin (Alb), globulin (Glob), urea, creatinine (Cr), creatine kinase (CK), lactate, fibrinogen, glucose, cortisol, total and ionized calcium (Ca^{2+}), serum inorganic phosphate (SIP), chloride (Cl^{-}), potassium (K^{+}), sodium (Na^{+}) and magnesium (Mg^{+}).

2. Group 2:

   The same data were collected for Group 2, with additional data relating to the injuries, treatments and final outcomes which included (Appendix A):

   - History: Rhino factors: health prior to poaching event.
     Poaching factors: poaching method, type of weapon used and estimated time from poaching to veterinary attendance.
   - Clinical exam: Detailed description of wounds and other injuries sustained and classified according to severity (number, character) and other abnormal findings.
   - Treatment: Drugs used and doses.
   - Outcome: Survival (duration) or death.

Due to logistical reasons, such as time between collection and laboratory analysis, blood-gas analysis and anion gap determination was not evaluated in this study.

In cases where an injured rhino was visited on more than one occasion by the veterinarian, serial blood samples and observational plus clinical data were collected to allow longitudinal monitoring of the patient.

2.6 Statistical Analysis

All data were recorded in commercial spreadsheets using Excel 2013 (Microsoft, Redmond, WA 98052-6399, USA). Raw data were then imported into STATA 13 (StataCorp LP, 4905 Lakeway Drive, College Station, Texas 77845-4512, USA), by which all statistical analysis was done.

Where serial samples were collected from rhinos, only data from the first visit were used for comparisons between the two groups. The non-injured group (Group 1) was compared to the injured group (Group 2) for all haematological and biochemical measurands. Within Group 2, survivors and non-survivors were also compared.
Data from each measurand were formally assessed for normality using the Shapiro-Wilk W test. In cases where the measurand had a parametric distribution, the Two-Sample t-test with equal variance was used to compare mean values. Where data were non-parametrically distributed, the Two-Sample Wilcoxon rank-sum (Mann-Whitney) test was used to compare median values. Statistical significant differences between the compared groups were considered at a *P*-value < 0.05.

Reference intervals were determined from the non-injured rhinos (Group 1) in line with ASVCP guidelines (49, 50) and calculated using the Reference Value Advisor (Ref Val) version 2.1 (51). After the descriptive statistics were analysed, the data were presented in the form of histograms and inspected visually. Normality was assessed using the Anderson-Darling test and the McWilliams runs test was performed to evaluate symmetry. The Box-Cox method was used to transform non-Gaussian data. Reference intervals were determined preferentially using the robust method, but where not appropriate, the non-parametric method was used to calculate the upper and lower reference values. This method of determining reference intervals used here, has also been described and used previously (32,52).

The ADVIA and Cell-Dyn analysers were compared using the results of paired measurements of a quality control material with an assigned target mean. Seven measurements were performed over a period of 7 months from different samples. Results were compared firstly using a Wilcoxon signed-rank test, with significance set at *P*-value <0.05. Secondly, the mean bias was calculated for each measurand, with the assigned mean for the ADVIA used as the target mean. The resulting bias for the Cell-Dyn was compared to the

laboratory's total allowable error goals for haematology. The paired sample comparison revealed significant differences for MCHC, Hct, MCV and RDW. Bias exceeded analytical quality goals for these same measurands. The results from rhinos were therefore not grouped together for these measurands, but were for RBC, WBC, HGB and thrombocytes.

The results for both haematology and serum biochemistry measurands were tabulated (Tables 3.4 – 3.7) showing descriptive statistics including mean, median, standard deviation, standard error of the mean, minimum, maximum, 95% confidence interval, reference interval and *P*-value. Results were listed for the two groups as well as within the injured group, the two sub-groups being Survivors and Non-survivors.

Chapter 3: Results

3.1 Study Population:

The study population consisted of a total of 74 individual southern white rhinos spread throughout South Africa on both private as well as government owned land. The healthy control group (Group 1) (n = 48) consisted of 20 males and 28 females. The injured study group (Group 2) (n = 26) consisted of 18 males and eight females.

When looking at life stages, the control group included 33 adults, seven sub-adults and eight calves while the injured group had 23 adults, one sub-adult and two calves.

The sex and life stage distribution of Groups 1 (control) and 2 (injured) are given in Table 3.1. Clinical information as well as some signalment information about the rhinos included in Group 2 (Injured) is given in Table 3.2. The effect of age and sex on the measurands were not evaluated in this study.

Due to poor compliance from many private practitioners in sending samples from injured rhinos to the laboratory, physical and clinical examination data as well as time period scores referred to in Table 2.1 were often lacking. Due to the low numbers of complete data sets, it was decided to not include these results nor discuss them in this dissertation since any conclusions would be baseless.

Table 3.1: Summary of white rhino sex and life stage distribution between Group 1 (Control) and Group 2 (Injured):

Sex	Group 1 (n = 48), (%)	Group 2 (n = 26), (%)
Male	20 (41.7)	18 (69.2)
Female	28 (58.3)	8 (30.8)
Life stage		
Calf	8 (16.6)	2 (8.7)
Sub-adult	7 (14.6)	1 (4.3)
Adult	33 (68.8)	23 (88.5)

Table 3.2: Clinical findings as well as signalment of the rhinos included in Group 2 (Injured):

Project ID	Province	Sex	Life Stage	Survivor	Clinical findings
001	Western Cape	Male	Adult	Yes	Horns removed, cutting into face
002	Western Cape	Female	Adult	Yes	Horns removed, cutting into face
003	North West	Male	Adult	Yes	Poaching victim, bullet wounds, location of injury unknown.
004	North West	Male	Adult	Yes	Extent of injury unknown, but poaching related
005	Limpopo	Male	Calf	Yes	Severe laceration, horn wound on ventral abdomen from inter-species aggression.
006	Limpopo	Female	Adult	Yes	Bullet wound, left carpus.
008	Limpopo	Male	Sub-adult	Yes	Chronic bullet wound, right hind limb (arthroscopy wound)
009	Limpopo	Male	Adult	Yes	Wounds on caudal aspect of front limbs, from attempted escape from enclosure
010	Gauteng	Female	Adult	No	Bullet wound to dorsal cervical vertebra. Mild right hind limb lameness. Severe machete wound on middle and right dorsal lumbar region.
011	Gauteng	Male	Adult	Yes	Bullet wound to right scapular region.
012	Limpopo	Male	Adult	Yes	Poaching victim, bullet wounds
013	Limpopo	Female	Adult	Yes	Poaching victim, bullet wounds
014	Mpumalanga	Male	Adult	Yes	Bullet wound to face, dorsal to right eye. Unilateral nasal discharge with mild epistaxis.
024	Kwa-Zulu Natal	Female	Adult	Yes	Poaching related bullet wounds.

Table 3.2 (continued): Clinical findings as well as signalment of the rhinos included in Group 2 (Injured):

Project ID	Province	Sex	Life Stage	Survivor	Clinical findings
032	North West	Male	Adult	Yes	Bullet wound, level of left elbow joint. Intermittently weight bearing.
041	Limpopo	Male	Calf	No	Severe poaching related injuries. Severe muscle damage.
043	North West	Male	Adult	Yes	Bullet wound, dorsal frontal sinus area. Unilateral epistaxis, no other clinical findings.
056	Limpopo	Female	Adult	No	Bullet wound to carpal region including fracture.
057	Limpopo	Male	Adult	Yes	Poaching related bullet wounds to flank.
058	Limpopo	Male	Adult	Yes	Poaching related bullet wounds to dorsal neck.
059	Limpopo	Male	Adult	No	Severe bullet wound to dorsal cervical vertebrae spinous processes and affecting nuchal ligament.
060	Eastern Cape	Female	Adult	Yes	Severe facial wounds, both horns and much of maxilla removed, sinuses exposed.
061	North West	Male	Adult	Yes	Horns removed, wounds granulating already.
062	Limpopo	Male	Adult	No	Poaching related bullet wounds.
063	Free State	Male	Adult	Yes	Poaching related bullet wounds, right side of the neck in the hump.
064	Free State	Female	Adult	No	Right proximal humerus and scapula fracture due to bullet wound.

3.2 Haematology:

Results for haematology measurands are shown in Tables 3.3 – 3.5. The Hct, MCV, MCHC and RDW (included in Tables 3.4 and 3.5) that were measured by the two different analysers were listed and analysed separately (as per reasons discussed in Chapter 2).
None of the non-survivors in the injured group were analysed on the Cell-Dyn, therefore no comparison was done between the two sub-groups for this analyser.

To evaluate the effect of injury, haematology results from 36 non-injured rhinos were compared with results from 23 injured rhinos, with the exception of differential white cell counts, where the results from only 20 injured rhinos were available. Descriptive statistics as well as reference intervals for each haematology measurand are shown in Tables 3.3 – 3.5.

With regards to parasite identification, *Theileria* sp. was found in two individual rhinos from the control group and in five individuals from the injured group.

3.2.1 Erythrocytes:

The MCH was lower (*P*-value = 0.03) in the non-survivors compared to the survivors categories within the injured group (Table 3.3). For the four measurands that could not be pooled, none were significantly different between the groups for the Cell-Dyn analyser (Table 3.4). However, on the ADVIA analyser, both the Haematocrit (*P*-value = 0.03) and MCV (*P*-value = 0.04) were lower in the non-survivors compared to the survivors of the injured group (Table 3.5).
On blood smear examination, 31/36 of the rhino blood smears evaluated had Heinz bodies.

3.2.2 Leukocytes:

The total white blood cell count was higher (*P*-value = 0.03) for the injured group compared to the control group (Table 3.3). Neutrophils were the predominant white blood cell type with segmented neutrophil numbers being significantly higher (*P*-value = 0.0003) in the injured group compared to the non-injured group (Table 3.3). The eosinophil count was significantly lower (*P*-value = 0.0001) in the injured group compared to the non-injured group (Table 3.3). Lymphocyte counts were both significantly lower (*P*-value < 0.0001 and *P*-value = 0.02 respectively) in injured animals compared to non-injured animals (Table 3.3) as well as the non-survivors compared to the survivors of the injured group (Table 3.3). Basophil counts are not shown in Table 3.4 since all individuals except one had a count of 0.0 x 10^9/L.

3.2.3 Thrombocytes:

The thrombocyte count was significantly (*P*-value = 0.0001) higher in the injured group, compared to the non-injured group (Table 3.3). There was a tendency towards the count being higher in non-survivors when compared to survivors (Table 3.3).
Mild to moderate platelet aggregation was found in 68% (40/59) of samples in this study.

Table 3.3: Comparison of haematology measurands of white rhinos between Group 1 (Control) and Group 2 (Injured), as well as within Group 2, distinguishing between survivors and non-survivors.

Measurand[a]	Groups	n	Mean	Median	SD	SEM	Min.	Max.	95% CI (mean)	Reference Interval	*P-value*
RCC ($x10^{12}$/L)	*Control*	36	8.01	8.10	1.13	0.19	4.79	10.54	7.62 - 8.39	6.0 - 10.2	0.11
	Injured	23	7.57	7.40	0.82	0.17	6.26	9.71	7.21 - 7.92		
	Injured: Survivors	19	7.63	7.42	0.83	0.19	6.39	9.71	7.23 - 8.03		0.41
	Injured: Non-survivors	4	7.26	7.30	0.78	0.39	6.26	8.17	6.01 - 8.50		
Haemoglobin (g/L)	*Control*	36	167.92	170.50	18.26	3.04	110.00	196.00	161.74 - 174.10	137.5 - 201.6	0.09
	Injured	23	158.39	164.00	23.05	4.81	104.00	200.00	148.42 - 168.39		
	Injured: Survivors	19	162.89	166.00	20.45	4.69	118.00	200.00	153.04 - 172.75		0.07
	Injured: Non-survivors	4	137.00	139.00	25.47	12.73	104.00	166.00	96.47 - 177.53		
MCH (pg)	*Control*	36	21.15	21.10	1.89	0.31	16.00	24.40	20.51 - 21.79	17.5 - 25.0	0.67
	Injured	23	20.93	21.20	2.22	0.46	16.60	25.00	19.96 - 21.89		
	Injured: Survivors	19	21.37	21.50	1.90	0.43	18.40	25.00	20.45 - 22.28		0.03*
	Injured: Non-survivors	4	18.83	18.00	2.75	1.37	16.60	22.70	14.46 - 23.19		
WCC ($x10^9$/L)	*Control*	36	10.47	10.16	1.90	0.32	5.19	14.24	9.83 - 11.11	7.8 - 14.8	0.03*
	Injured	23	12.55	11.20	4.28	0.89	6.07	23.39	10.70 - 14.40		
	Injured: Survivors	19	12.23	11.20	3.74	0.86	6.07	20.30	10.14 - 14.03		0.87
	Injured: Non-survivors	4	14.07	12.09	6.82	3.41	8.70	23.39	3.21 - 24.92		
Segmented Neutrophils ($x10^9$/L)	*Control*	36	3.95	3.62	1.58	0.26	1.39	7.56	3.42 - 4.49	0.7 - 7.3	0.0003*
	Injured	20	6.61	6.32	3.15	0.70	1.58	15.43	5.13 - 8.08		
	Injured: Survivors	17	6.80	7.17	3.38	0.82	1.58	15.43	5.06 - 8.53		0.31
	Injured: Non-survivors	3	5.52	6.09	1.01	0.58	4.35	6.11	3.01 - 8.03		
Band Neutrophils ($x10^9$/L)	*Control*	36	1.02	0.80	0.97	0.16	0.00	4.13	0.69 - 1.34	0 - 3.4	0.98
	Injured	20	1.72	0.62	2.71	0.61	0.00	10.53	0.45 - 2.98		
	Injured: Survivors	17	1.41	0.52	2.67	0.65	0.00	10.53	0.032 - 2.78		0.12
	Injured: Non-survivors	3	3.47	3.98	2.64	1.52	0.61	5.81	0.00 - 10.02		

n = number of samples; SD = standard deviation; SEM = standard error of the mean; Min. = Minimum; Max. = Maximum; 95% CI = 95% Confidence interval.
[a] RCC = Red blood cell count; MCH = mean corpuscular haemoglobin; WCC = white blood cell count
* = *P*-value < 0.05

Table 3.3 (continued): Comparison of haematology measurands of white rhinos between Group 1 (Control) and Group 2 (Injured), as well as within Group 2, distinguishing between survivors and non-survivors.

Measurand	Groups	n	Mean	Median	SD	SEM	Min.	Max.	95% CI (mean)	Reference Interval	*P-value*
Lymphocytes ($x10^9$/L)	*Control*	36	3.49	3.42	1.21	0.20	1.56	8.10	3.08 - 3.90	2.1 - 7.0	<0.0001*
	Injured	20	2.08	2.23	1.11	0.25	0.46	4.63	1.56 - 2.59		
	Injured: Survivors	17	2.30	2.50	1.05	0.26	0.61	4.63	1.76 - 2.84		0.02*
	Injured: Non-survivors	3	0.82	0.96	0.31	0.18	0.46	1.04	0.04 - 1.60		
Monocytes ($x10^9$/L)	*Control*	36	1.07	1.00	0.49	0.08	0.31	2.42	0.91 - 1.24	0.3 - 2.3	0.42
	Injured	20	1.38	1.07	1.03	0.23	0.26	4.53	0.90 - 1.86		
	Injured: Survivors	17	1.45	1.09	1.09	0.26	0.26	4.53	0.89 - 2.01		0.56
	Injured: Non-survivors	3	1.00	1.04	0.52	0.30	0.46	1.49	0.00 - 2.28		
Eosinophils ($x10^9$/L)	*Control*	36	0.94	0.97	0.57	0.10	0.00	2.67	0.74 – 1.13	0 - 2.3	0.0001*
	Injured	20	0.40	0.12	0.64	0.14	0.00	2.57	0.10 - 0.69		
	Injured: Survivors	17	0.44	0.13	0.68	0.17	0.00	2.57	0.09 - 0.79		0.44
	Injured: Non-survivors	3	0.15	0.00	0.26	0.15	0.00	0.45	0.00 - 0.80		
Thrombocytes ($x10^9$/L)	*Control*	36	382.31	363.00	105.54	17.59	194.00	729.00	346.59 - 418.01	215.9 - 658.8	0.0001*
	Injured	23	562.78	497.00	233.98	48.79	325.00	1204.00	461.60 - 663.96		
	Injured: Survivors	19	534.47	437.00	226.09	51.86	325.00	1204.00	425.50 - 643.44		0.06
	Injured: Non-survivors	4	697.25	600.00	256.12	128.05	518.00	1071.00	289.71 - 1104.78		

n = number of samples; SD = standard deviation; SEM = standard error of the mean; Min. = Minimum; Max. = Maximum; 95% CI = 95% Confidence interval.
* = *P*-value < 0.05

Table 3.4: Comparison between Group 1 (Control) and 2 (Injured) of measurands from the Cell-Dyn analyser that could not be pooled with results from the ADVIA analyser.

Measurand [b]	Groups	n	Mean	Median	SD	SEM	Min.	Max.	95% CI (mean)	Reference Interval	*P-value*
Ht (L/L)	*Control*	21	0.45	0.45	0.05	0.01	0.32	0.51	0.424 - 0.470	0.3 - 0.6	0.49
	Injured	6	0.46	0.46	0.06	0.02	0.39	0.53	0.404 - 0.522		
MCV (fL)	*Control*	21	58.00	57.90	5.56	1.21	43.70	66.90	55.47 - 60.53	43.9 - 68.1	0.15
	Injured	6	61.80	59.30	5.38	2.19	56.90	69.40	56.16 - 67.44		
MCHC (g/dL)	*Control*	21	36.27	36.20	0.81	0.18	34.20	37.90	35.90 - 36.64	34.3 - 37.8	0.45
	Injured	6	35.93	35.80	1.41	0.57	34.60	38.00	34.46 - 37.41		
RDW (%)	*Control*	21	24.59	24.30	2.24	0.49	20.80	29.00	23.56 - 25.61	20.5 - 30.1	0.86
	Injured	6	24.42	24.20	1.04	0.42	23.30	25.80	23.33 - 25.51		

n = number of samples; SD = standard deviation; SEM = standard error of the mean; Min. = Minimum; Max. = Maximum; 95% CI = 95% Confidence interval.
[b] Ht = Haematocrit; MCV = Mean corpuscular volume; MCHC = mean corpuscular haemoglobin concentration; RDW = red cell distribution width.

Table 3.5: Comparison between Group 1 (Control) and 2 (Injured) of measurands from the ADVIA analyser that could not be pooled with results from the Cell-Dyn analyser.

Measurand [b]	Groups	n	Mean	Median	SD	SEM	Min.	Max.	95% CI (mean)	Reference Interval	*P-value*
Ht (L/L)	*Control*	15	0.51	0.51	0.04	0.01	0.44	0.56	0.484 - 0.526	0.4 - 0.6	0.06
	Injured	17	0.46	0.48	0.06	0.02	0.31	0.56	0.429 - 0.495		
	Injured: Survivors	13	0.48	0.48	0.05	0.01	0.37	0.56	0.449 - 0.512		0.03*
	Injured: Non-survivors	4	0.40	0.41	0.07	0.03	0.31	0.48	0.289 - 0.511		
MCV (fL)	*Control*	15	59.85	63.70	7.09	1.83	44.50	67.40	55.93 - 63.78	46.4 - 81.4	0.58
	Injured	17	60.91	62.50	7.22	1.75	49.80	72.10	57.19 - 64.62		
	Injured: Survivors	13	62.75	63.60	6.32	1.75	53.90	72.10	58.92 - 66.57		0.04*
	Injured: Non-survivors	4	54.93	52.10	7.48	3.74	49.80	65.70	43.02 - 66.83		
MCHC (g/dL)	*Control*	15	34.92	34.90	1.73	0.45	30.80	37.40	33.96 - 35.88	30.0 - 38.0	0.07
	Injured	17	33.75	33.90	2.33	0.56	25.60	36.20	32.56 - 34.95		
	Injured: Survivors	13	33.60	33.90	2.65	0.73	25.60	36.20	32.00 - 35.20		0.57
	Injured: Non-survivors	4	34.25	34.50	0.64	0.32	33.30	34.70	33.23 - 35.27		
RDW (%)	*Control*	15	20.40	20.40	0.80	0.21	19.30	22.00	19.96 - 20.84	18.6 - 22.1	0.95
	Injured	17	20.38	20.40	1.13	0.27	18.00	22.80	19.80 - 20.96		
	Injured: Survivors	13	20.24	20.40	0.75	0.21	18.80	21.40	19.78 - 20.69		0.38
	Injured: Non-survivors	4	20.83	21.25	2.04	1.02	18.00	22.80	17.58 - 24.07		

n = number of samples; SD = standard deviation; SEM = standard error of the mean; Min. = Minimum; Max. = Maximum; 95% CI = 95% Confidence interval.
[b] Ht = Haematocrit; MCV = Mean corpuscular volume; MCHC = mean corpuscular haemoglobin concentration; RDW = red cell distribution width. * = *P*-value < 0.05

3.3 Serum biochemistry:

In order to evaluate the effects of injury, serum biochemistry results from 43 non-injured rhinos were compared with results from 25 injured rhinos. Reference intervals together with all the descriptive statistics for each measurand is shown in Table 3.6.

3.3.1 Evaluation of serum and plasma proteins:

There was a tendency for total serum proteins to be lower among non-survivors. Both albumins and globulins were similar between the groups. However, fibrinogen was higher (*P*-value = 0.008) in the injured group, compared to the controls (Table 3.6)

3.3.2 Evaluation of the urea and creatinine:

Only urea measured higher (*P*-value = 0.04) in the injured group when compared to the non-injured group (Table 3.6). Creatinine showed no difference between groups.

3.3.3 Evaluation of electrolytes and selected minerals:

Among the electrolytes, sodium, potassium and chloride were not different between the groups. Magnesium, total calcium, ionized calcium and phosphate levels were all lower (*P*-value = 0.002, *P*-value = 0.004, *P*-value = 0.04 and *P*-value = 0.007 respectively) in the injured group, compared to the non-injured group. Magnesium was also lower (*P*-value = 0.03) in the non-survivors, compared to the survivors.

3.3.4 Evaluation of the liver:

Only AST was increased in the injured group (*P*-value = 0.005), while ALP and GGT showed no differences between groups. Although not significant (*P*-value = 0.11), GLDH tended to be higher in the injured group compared to the non-injured group, but within the injured group, levels tended to be lower among non-survivors (Table 3.6).

3.3.5 Detection of muscle injury:

Two enzymes measured in this study are good indicators of muscle injury when looked at in conjunction, and these are CK and AST. It is important to note that AST is not only specific to muscle injury but also liver damage. Animals in the injured group had higher CK and AST (*P*-value = 0.0001 and *P*-value = 0.005 respectively), but there was no difference between the rhinos that survived and those that didn't (Table 3.6).

3.3.6 Evaluation of some metabolic and endocrine measurands:

Lactate was measured in serum from both normal serum tubes as well as fluoride oxalate tubes. Fluoride oxalate inhibits glycolysis and thereby prevents any additional lactate production in the tube after sample collection. Differences were reported between the different tubes, with the lactate from the serum tube from the non-injured animals having a median of 9.2 mmol/L versus the median of 5.2 mmol/L in the fluoride oxalate tube. There were no significant differences for lactate measurements between the different groups for either of the tubes. However, there was a tendency for lactate to be higher in the injured group compared to the non-injured group while there was a tendency for lactate to be lower in non-survivors compared to survivors.

Glucose was higher (*P*-value = 0.04) in the injured group compared to the non-injured group (Table 3.6). Cortisol levels were also higher (*P*-value = 0.03) in the injured group compared to the non-injured group (Table 3.6).

Table 3.6: Comparison of serum biochemistry measurands of white rhinos between Group 1 (Control) and Group 2 (Injured), as well as within Group 2, distinguishing between survivors and non-survivors.

Measurand [c]	Groups	n	Mean	Median	SD	SEM	Min.	Max.	95% CI (mean)	Reference Interval	*P-value*
TSP (g/L)	*Control*	43	85.3	86.3	6.5	0.99	73.3	98.8	83.27 - 87.28	71.8 - 98.8	0.51
	Injured	25	82.5	84.0	12.2	2.45	54.3	102.0	77.4 - 87.50		
	Injured: Survivors	19	84.1	86.0	11.5	2.7	54.3	102.0	78.53 - 89.66		0.28
	Injured: Non-survivors	6	77.3	74.6	14.0	5.7	56.1	95.9	62.56 - 91.97		
Albumin (g/L)	*Control*	43	28.9	28.8	4.7	0.7	21.0	40.1	27.49 - 30.36	21 - 39.9	0.16
	Injured	25	27.1	28.8	5.9	1.2	14.9	37.3	24.69 - 29.52		
	Injured: Survivors	19	27.8	29.1	5.7	1.3	17.4	37.3	25.10 - 30.57		0.27
	Injured: Non-survivors	6	24.8	26.0	6.4	2.6	14.9	32.5	18.11 - 31.45		
Globulins (g/L)	*Control*	43	56.4	57.0	6.3	1.0	41.5	70.0	54.42 - 58.30	41.8 - 69.8	0.65
	Injured	25	55.4	54.9	11.6	2.3	35.3	81.0	50.59 - 60.13		
	Injured: Survivors	19	56.3	56.4	10.0	2.3	35.3	71.1	51.45 - 61.10		0.50
	Injured: Non-survivors	6	52.5	48.6	16.3	6.7	35.9	81.0	35.35 - 69.62		
ALP (U/L)	*Control*	43	83.9	76.0	35.8	5.47	39.0	227.0	72.92 - 94.99	39.2 - 165.9	0.28
	Injured	24	90.1	86.0	35.8	7.31	43.0	183.0	74.96 - 105.21		
	Injured: Survivors	18	94.7	90.5	38.3	9.0	43.0	183.0	75.62 - 113.71		0.35
	Injured: Non-survivors	6	76.3	79.5	24.8	10.1	47.0	113.0	50.29 - 102.38		
AST (U/L)	*Control*	43	60.0	54.0	18.7	2.9	29.0	104.0	54.28 - 65.76	29.2 - 103.6	0.005*
	Injured	25	178.7	80.0	278.2	55.6	24.0	1293.0	63.89 - 293.55		
	Injured: Survivors	19	146.7	80.0	172.9	39.7	39.0	774.0	63.39 - 230.01		0.82
	Injured: Non-survivors	6	280.2	82.5	497.6	203.2	24.0	1293.0	0.00 - 802.37		
GGT (U/L)	*Control*	43	14.0	14.0	3.1	0.5	8.0	21.0	13.02 - 14.93	7.6 - 20.2	0.17
	Injured	24	12.7	12.0	4.6	0.9	6.0	26.0	10.73 - 14.61		
	Injured: Survivors	18	12.9	12.5	4.9	1.2	6.0	26.0	10.52 - 15.37		0.62
	Injured: Non-survivors	6	11.8	11.0	3.9	1.6	8.0	17.0	7.72 - 15.95		

n = number of samples; SD = standard deviation; SEM = standard error of the mean; Min. = Minimum; Max. = Maximum; 95% CI = 95% Confidence interval.
[c] TSP = total serum protein; ALP = alkaline phosphatase; AST = aspartate aminotransferase; GGT = γ-glutamyltransferase
* = *P*-value < 0.05

Table 3.6 (continued): Comparison of serum biochemistry measurands of white rhinos between Group 1 (Control) and Group 2 (Injured), as well as within Group 2, distinguishing between survivors and non-survivors.

Measurand [c]	Groups	n	Mean	Median	SD	SEM	Min.	Max.	95% CI (mean)	Reference Interval	*P-value*
GLDH (U/L)	*Control*	43	3.6	3.0	2.0	0.3	1.0	11.0	2.88 - 4.14	1.0 - 10.8	0.11
	Injured	24	4.3	4.0	2.5	0.5	0.0	12.0	3.29 - 5.39		
	Injured: Survivors	18	4.9	4.1	2.5	0.6	2.0	12.0	3.55 - 6.03		0.11
	Injured: Non-survivors	6	3.0	3.0	2.1	0.9	0.0	6.0	0.80 - 5.20		
CK (U/L)	*Control*	43	229.7	192.0	104.1	15.9	103.0	444.0	197.63 - 261.71	103.3 - 442.4	0.0001*
	Injured	25	5393.5	1219.0	10842.8	2168.5	65.0	40230.0	917.80 - 9869.16		
	Injured: Survivors	19	4491.1	1219.0	9220.6	2115.3	94.0	40230.0	46.89 - 8935.22		0.80
	Injured: Non-survivors	6	8251.2	1855.0	15663.8	6394.7	65.0	40000.0	0.00 - 24689.3		
Urea (mmol/L)	*Control*	43	3.7	3.2	1.6	0.24	1.8	7.8	3.18 - 4.16	1.8 - 7.7	0.04*
	Injured	25	5.6	4.2	3.7	0.74	1.8	16.3	4.02 - 7.09		
	Injured: Survivors	19	5.7	4.2	3.7	0.8	2.4	16.3	3.92 - 7.46		0.52
	Injured: Non-survivors	6	5.1	3.0	4.2	1.7	1.8	12.1	0.74 - 9.52		
Creatinine (umol/L)	*Control*	43	132.7	137.0	33.9	5.2	66.0	202.0	122.32 - 143.17	64.7 - 197.5	0.60
	Injured	25	128.8	128.0	32.2	6.4	69.0	187.0	115.59 - 142.11		
	Injured: Survivors	19	128.3	128.0	29.5	6.8	69.0	176.0	114.12 - 142.51		0.89
	Injured: Non-survivors	6	130.5	126.5	42.8	17.5	74.0	187.0	85.55 - 175.45		
Sodium (mmol/L)	*Control*	48	131.2	131.0	3.7	0.53	121.0	141.0	130.16 - 132.30	123.6 - 138.7	0.62
	Injured	24	130.8	131.0	3.3	0.67	122.0	137.0	129.40 - 132.17		
	Injured: Survivors	19	131.3	131.0	2.9	0.7	125.8	137.0	129.92 - 132.69		0.13
	Injured: Non-survivors	5	128.8	128.0	4.3	1.9	122.0	134.0	123.43 - 134.16		
Potassium (mmol/L)	*Control*	48	5.8	4.8	2.6	0.4	4.0	16.4	5.03 - 6.56	3.9 - 6.3	0.96
	Injured	24	5.4	5.0	1.4	0.3	3.7	9.6	4.79 - 5.97		
	Injured: Survivors	19	5.2	4.9	1.0	0.2	3.9	7.2	4.70 - 5.64		0.50
	Injured: Non-survivors	5	6.2	5.1	2.4	1.1	3.7	9.6	3.15 - 9.19		

n = number of samples; SD = standard deviation; SEM = standard error of the mean; Min. = Minimum; Max. = Maximum; 95% CI = 95% Confidence interval.
[c] GLDH = glutamate dehydrogenase; CK = Creatine kinase
* = *P*-value < 0.05

Table 3.6 (continued): Comparison of serum biochemistry measurands of white rhinos between Group 1 (Control) and Group 2 (Injured), as well as within Group 2, distinguishing between survivors and non-survivors.

Measurand[c]	Groups	n	Mean	Median	SD	SEM	Min.	Max.	95% CI (mean)	Reference Interval	*P-value*
Chloride (mmol/L)	*Control*	43	90.3	90.1	3.2	0.5	84.1	89.0	89.27 - 91.232	83.7 - 96.8	0.22
	Injured	23	91.2	91.0	2.6	0.5	86.4	97.1	90.10 - 92.32		
	Injured: Survivors	18	91.0	91.0	2.6	0.6	86.4	97.1	89.68 - 92.25		0.40
	Injured: Non-survivors	5	92.1	93.0	2.6	1.1	89.0	95.4	88.93 - 95.27		
Magnesium (mmol/L)	*Control*	43	1.16	1.15	0.11	0.02	0.95	1.45	1.12 - 1.19	1.0 - 1.4	0.002*
	Injured	23	1.01	0.95	0.24	0.05	0.73	1.70	0.91 - 1.12		
	Injured: Survivors	18	1.07	1.06	0.24	0.06	0.74	1.70	0.95 - 1.19		0.03*
	Injured: Non-survivors	5	0.81	0.84	0.05	0.02	0.73	0.85	0.74 - 0.87		
Total Calcium (mmol/L)	*Control*	43	2.98	2.98	0.12	0.02	2.74	3.31	2.94 - 3.02	2.7 - 3.3	0.004*
	Injured	23	2.91	2.88	0.54	0.11	2.14	5.18	2.68 - 3.15		
	Injured: Survivors	18	2.98	2.89	0.58	0.14	2.14	5.18	2.69 - 3.27		0.09
	Injured: Non-survivors	5	2.69	2.66	0.31	0.14	2.41	3.22	2.30 - 3.08		
Ionized Calcium (mmol/L)	*Control*	39	1.47	1.46	0.07	0.01	1.26	1.65	1.44 - 1.49	1.3 - 1.6	0.04*
	Injured	17	1.41	1.41	0.11	0.03	1.18	1.61	1.36 - 1.47		
	Injured: Survivors	13	1.44	1.43	0.11	0.03	1.18	1.61	1.37 - 1.50		0.06
	Injured: Non-survivors	4	1.33	1.36	0.09	0.05	1.21	1.41	1.19 - 1.48		
SIP (mmol/L)	*Control*	43	1.5	1.5	0.4	0.06	0.7	2.3	1.39 - 1.63	0.7 - 2.3	0.007*
	Injured	24	1.2	1.1	0.5	0.1	0.4	2.2	0.99 - 1.43		
	Injured: Survivors	18	1.2	1.1	0.4	0.1	0.6	2.2	0.99 - 1.43		0.96
	Injured: Non-survivors	6	1.2	1.1	0.7	0.3	0.4	2.1	0.433 - 1.97		

n = number of samples; SD = standard deviation; SEM = standard error of the mean; Min. = Minimum; Max. = Maximum; 95% CI = 95% Confidence interval.
[c] = SIP = serum inorganic phosphate
* = *P*-value < 0.05

Table 3.6 (continued): Comparison of serum biochemistry measurands of white rhinos between Group 1 (Control) and Group 2 (Injured), as well as within Group 2, distinguishing between survivors and non-survivors.

Measurand	Groups	n	Mean	Median	SD	SEM	Min.	Max.	95% CI (mean)	Reference Interval	*P-value*
Cortisol (nmol/L)	*Control*	34	36.1	33.5	22.0	3.8	6.0	114.0	28.45 - 43.79	7.2 - 97.2	0.03*
	Injured	15	77.5	66.2	63.4	16.4	9.0	210.0	42.36 - 112.58		
	Injured: Survivors	11	62.1	49.9	45.7	13.8	13.0	153.0	31.36 - 92.77		0.30
	Injured: Non-survivors	4	119.8	130.2	92.4	46.2	9.0	210.0	0.00 - 266.81		
****Lactate - Serum** (mmol/L)	*Control*	43	9.2	9.2	4.0	0.60	2.5	18.6	8.01 - 10.44	2.7 - 19.2	0.76
	Injured	25	10.3	8.7	7.2	1.43	2.4	26.7	7.38- 13.29		
	Injured: Survivors	19	10.9	8.8	7.4	1.7	3.1	26.7	7.37 - 14.51		0.25
	Injured: Non-survivors	6	8.4	6.0	6.5	2.6	2.4	19.3	1.61-15.23		
****Lactate - Fluoride Oxalate** (mmol/L)	*Control*	42	6.5	5.2	4.2	0.7	1.0	17.2	5.21-7.83	0.9 - 19	0.64
	Injured	21	7.1	3.4	6.6	1.4	1.2	23.3	4.06 - 10.10		
	Injured: Survivors	16	8.4	6.6	7.1	1.8	1.2	23.3	4.66 - 12.22		0.14
	Injured: Non-survivors	5	2.7	3.3	0.8	0.4	1.8	3.4	1.68 - 3.76		
Glucose (mmol/L)	*Control*	34	6.5	6.4	1.7	0.3	2.6	10.1	5.92 - 7.10	3.4 - 9.9	0.04*
	Injured	11	8.0	8.3	2.9	0.9	3.2	12.1	6.06 - 9.92		
	Injured: Survivors	6	9.3	9.5	1.4	0.6	7.2	10.7	7.73 - 10.76		0.12
	Injured: Non-survivors	5	6.5	4.7	3.6	1.6	3.2	12.1	2.03 - 10.93		
Fibrinogen (g/L)	*Control*	31	3.8	3.8	0.7	0.1	1.5	4.9	3.55 - 4.04	1.57 - 5.01	0.008*
	Injured	14	5.0	4.7	1.5	0.4	2.1	7.6	4.10 - 5.86		
	Injured: Survivors	10	4.9	4.6	1.7	0.5	2.1	7.6	3.65 - 6.11		0.57
	Injured: Non-survivors	4	5.2	5.2	1.0	0.5	4.2	6.3	3.58 - 6.87		

n = number of samples; SD = standard deviation; SEM = standard error of the mean; Min. = Minimum; Max. = Maximum; 95% CI = 95% Confidence interval.
* = *P*-value < 0.05
** = Lactate measured from both serum and fluoride oxalate tubes, showing effect of ongoing red cell metabolism in serum.

Chapter 4: Discussion

The white rhino population in South Africa is under tremendous pressure due to the international demand for rhino horn. Since no legal international trade in rhino horn exist, poaching of the animals in order to obtain horn illegally has dramatically increased since 2008. For the past three years more than a thousand white rhinos per year were poached to supply this demand. As a consequence of these poaching activities, some rhinos are injured and need veterinary care. Very little data are available on the haematology and serum biochemistry measurands of free roaming animals during this time, which prompted this study. Our focus was to determine reliable reference intervals for haematology and selected serum biochemistry measurands in healthy white rhinos and using these as a comparison for the values of injured white rhinos. Since the start of our study two other groups published baseline values and reference intervals for various haematological and biochemistry measurands in free roaming white rhinos (26, 32, 53, 54) and these findings are compared to ours below.

Differences found between the two main groups as well as the two sub-groups of the injured group in this study, were used as areas of focus for the discussion below. Regarding the sex ratios, a significantly higher number of injured males were recorded and sampled. Although the reason for this finding was not investigated, we are able to postulate on some possible reasons for this. It is possible that this is the true representation of poaching sex ratio, although bigger sample sizes would be needed to make any such conclusions. It is also possible that there were simply more males in the surveyed area of this study, or that males are more likely to survive poaching events. Another possibility is that females are more effectively killed during poaching because they tend to be found in more of a herd structure or would be trying to protect their young. Males would likely be found alone and thus could be more difficult targets, thus not being killed in as many attacks. It's also possible that males make more attractive targets for poachers since mature males would have bigger horns. Males are also territorial, and it could be that poachers come into contact with more males, simply by chance, because these males approach the invaders to their territory, and inadvertently become targets. What is interesting is that our finding seems to be similar to that seen in the Nepalese greater one-horned rhino (*Rhinoceros unicornis*), where the amount of males poached were double that of the females in 2017 (55).

When comparing results from the injured group with the normal healthy group, we found that total white blood cell count, segmented neutrophil count, thrombocyte count, fibrinogen, urea, AST, CK, glucose and cortisol were significantly higher while eosinophil and lymphocyte count, magnesium, calcium and phosphate were significantly lower. GLDH also tended to be higher in injured rhinos compared to healthy ones.

Upon comparing results from injured non-survivors with injured survivors, MCH, Ht, MCV, lymphocyte count and magnesium were significantly lower and GLDH had a tendency to also be lower in this sub-group. Thrombocyte count tended to be higher in non-survivors compared to survivors.

4.1 Haematology:

In a recent abstract by Hooijberg *et al.* published in conference proceedings (53), reference intervals for nine haematological measurands were published. All of these reference intervals compared well with reference intervals for the same measurands created in our study.

Furthermore, when comparing the means and medians for the haematology measurands of our study to the 95% confidence intervals of a recently published study by Miller *et al.* on a wild population of white rhinos (26), some measurands of our study were outside of the published confidence intervals. Red cell count and haemoglobin concentration of our study were higher while thrombocytes, total WBC count, neutrophils, monocytes, and eosinophils were lower. Plausible explanations for these differences relates to the homo– or heterogenicity of the populations, immobilisation protocols, handling of the samples, different haematology analysers used, and the health status of individuals in the study populations.

The control group of animals in this study originated from free living white rhinos across South Africa. Although free living, our control group included some individuals that were regularly receiving supplementary feeding as part of the farm management protocol. The animals from the Miller study (26) were free-living and completely wild, all originating from the same reserve. In our study, immobilisations were done by different clinicians each with their own combinations of drugs while all the rhinos in the Miller study (26) were immobilised with the same drug protocol.

Samples collected for this study were transported to the laboratory under varied conditions and were evaluated up to 24 hours after collection. In the Miller study (26), sample handling

was standardised, all samples were kept cool during transport and were all analysed within 6 hours of collection. The haematology analysers used in this study differed from the one used in the Miller study (26), which could also account for analytical differences between the studies for this species.

Although in both studies data were only collected from healthy animals, this health status was determined on physical examination which may not have detected animals with subtle underlying disease. The inadvertent inclusion of potential diseased animals may have skewed the results in both of the studies.

The method of immobilisation used has been shown to have differing effects on the haematology of red deer (56). Many animals in the control group of our study were accustomed to routine veterinary procedures such as dehorning, and also, in some cases, were exposed to human contact more often. The milder stress leucocytosis that we saw, compared to wild free ranging individuals from the Miller study (26) with no previous exposure to immobilisation from a helicopter, was therefore expected. The rhinos in our study were immobilised both from helicopters and from the ground and therefore some individuals would have been exposed to less stressors (ground darted animals) prior to blood collection. These factors could also have accounted for lower leukocyte counts and the milder stress leucocytosis seen in our study.

When activated, platelets form small clumps in the tube after blood collection in the EDTA tube (57-59). Our study had a high incidence of platelet aggregation on blood smears made at the time of analysis. Because blood smears were made from EDTA whole blood samples taken from auricular veins, the platelet aggregation seen on these smears are representative of platelet aggregation of the entire sample. This aggregation could cause a falsely decreased platelet count by the analyser (57, 58) which could be a reason for a relatively lower thrombocyte count in our study compared to those in the Miller study. None of the analysers used in these studies have been validated for use in the white rhino, and it is known that platelet counts are often inaccurate for various species on multiple haematology analysers (60-62).

Our reference intervals also differ from those of captive white rhinos published in the Species 360 database with respect to the following measurands: RBC count, Hb, total WBC count, monocyte, band neutrophil and lymphocyte absolute counts. For all of these measurands,

there was a tendency to have lower values in captive rhinos (23) compared to the free-ranging rhinos in our study, when means and medians were compared visually. The Species360 database's means for the abovementioned measurands were also lower than our study's determined 95% confidence intervals of the means for the healthy group. Similar differences in the red and white cell lineages were also seen in the Miller study compared to the Species360 database (26). In the Species360 database for captive white rhinos, very little metadata, method of immobilisation, sample handling or analysers used are available. Data that are entered into this database includes information from both sick and healthy zoo animals, which may cause differences in the haematology profiles compared to healthy wild individuals.

Theileria sp. infection has been shown in the majority of cases to be non-pathogenic with no clinically significant effect on the host (63) and is therefore seen as an incidental finding in the healthy white rhinos in our study. Pathogenic theileriosis has however been shown to affect red deer (*Cervus elaphus*) under conditions of severe translocation stress, disease and malnutrition (64). In black rhino, *Theileria bicornis* was also shown to be associated with mortality (65). Further investigation into the possible pathogenic role of *Theileria sp.* in white rhino is needed. Theileria was seen in more animals in the injured group. Whether this increase in incidence is clinically significant also requires further investigation.
The relative high prevalence of Heinz bodies has previously only been reported before in black rhinos (27, 66, 67). Our similar finding in the white rhino warrants further investigation on the erythrocytes, with particular focus on the significance of Heinz bodies, of this species.

Some of the haematology measurands of injured white rhinos differed from the normal values. The total white cell count was significantly elevated in injured rhinos. This elevation was due to an increase in neutrophils (both band and segmented), although only the levels of segmented neutrophils were statistically significant. Eosinophils and lymphocytes were both significantly decreased in the injured rhinos compared to non-injured ones. The lymphopaenia, eosinopaenia and mature neutrophilia are characteristics of a stress leukogram while the high neutrophil counts with a slight left shift are indicative of an inflammatory leucogram (68). All the injured rhinos had traumatic wounds which appeared infected. Since inflammation accompanies infection, the results are not surprising.

An interesting finding is the relative thrombocytosis in the injured compared to the control group. Thrombocytosis can be primary or secondary. Secondary thrombocytosis has been described as the most common form in humans, with tissue damage, infection and chronic inflammation accounting for 76% of the cases in one study (69). Taking into account that most injured rhinos, and certainly the non-survivors, had severe tissue damage and haemorrhage, a subsequent thrombocytosis was expected. Coagulopathies (bleeding tendency) have also been described related to trauma in other species (44, 70-73) and is generally a cause of platelet depletion. This loss of platelets from circulation is the likely stimulus for circulatory redistribution, which is a likely explanation for the thrombocyte increase as opposed to increased production (74). Thrombocytosis is also seen in iron deficient animals (74), which could be possible in those individuals that suffered severe haemorrhage. It is important to note that even though the increased thrombocyte count in the injured group is significantly higher than in the controls, the mean and median values still fall within the calculated reference interval. This fact is important to take into account when interpreting the clinical significance of such findings.

4.2 Serum Biochemistry:

The biochemistry measurands determined from the control group of this study were compared to all previously published baseline data for the white rhino and will be discussed sequentially.

Our means and reference intervals compared favourably with a study by van Heerden *et al.* in 1985 (11) for all measurands except CK, where our mean was on average 4 to 5 fold higher. Plausible explanations for the differences between these studies regarding CK include different methods of measurement as well as different immobilisation techniques used.

Reference intervals reported by Hooijberg *et al*. (53) compared very well with all our reported measurands.

Mathebula *et al.* (2012), published (25) baseline values from free-ranging white rhinos from the Kruger National Park (KNP), for selected biochemistry measurands. They also compared measurands from serum and plasma samples, and tested the effect of sex and life stage on these measurands (25). When comparing means and medians between their study and ours, total protein, albumin, globulin, calcium, urea, magnesium and inorganic phosphate compared well. Interestingly, our serum levels for ALP, AST and GGT were all higher than the serum

levels, but compared well with the plasma levels from their study. A higher mean (twofold) was also reported for CK in our study when compared to their study.
Of interest to note is that the Mathebula study reported significantly higher values for most measurands analysed from plasma, compared to serum. The exception being albumin that showed significantly higher levels in serum (25).

A more recent study by Hooijberg *et al*. (32), also using rhinos from the KNP, determined plasma reference intervals for selected biochemistry measurands while also comparing results from two different chemistry analysers (32). Like them we also used the Roche Cobas Integra analyser. Our reference intervals compare favourably with their results (32) with regards to total protein, CK, creatinine, GGT, phosphate, urea and glucose. This favourable comparison is despite our study using serum and theirs plasma. In our study globulins had lower reference interval values while albumin and AST had higher reference intervals values compared to theirs.

When comparing our serum biochemistry reference intervals, medians and means to the published data from captive white rhinos in the Species360 database (23), our results compare well for all measurands with only glucose having higher values reported in our study. Due to the lack of information within the Species360 database regarding signalment and husbandry conditions from these animals as well as information regarding the pre-analytical and analytical methods used with samples, it is difficult to theorise on the reason for only this difference in glucose concentrations. Drugs and dosage used for immobilisation of animals contained in the Species360 database is unknown and also not standardised within our study. Some immobilisation drugs are reported to cause hyperglycaemia (75-77). Another factor to consider is also that rhinos from our study were potentially more stressed before and during sample collection compared to captive populations. Stress induced hyperglycaemia is a known finding in many species (78).

The serum biochemistry measurands, as with haematology, of injured white rhinos differed from the normal values.
A recent study by Hooijberg *et al.* evaluated serum protein in wild white rhinos (healthy and injured) (54) and found that TSP, albumin and globulins to all have significantly lower concentrations in injured white rhinos. Our study does not share the same significant differences for these three measurands between the groups. For all three measurands, only

four injured individuals had levels below the determined reference intervals. However, there is a general downward trend comparing healthy and injured groups. Despite this downward trend, the medians of these measurands were still within the reference intervals for them. Our study differentiated between injured survivors and non-survivors where the medians for TSP, albumin and globulins of non-survivors were at the lower end of the reference intervals reported in the study by Hooijberg *et al.* (54). The heterogeneous nature of our study population in regards to nutritional (type, quality and quantity) and husbandry variables likely played a role in the lack of significant differences for these three measurands between healthy and injured groups. Differences in the type and severity of injuries sustained by the rhinos in our study compared to the Hooijberg *et al*. study (54) are speculated to be a possible cause in the dissimilarities seen regarding the significance of differences in TSP, albumin and globulin levels between healthy and injured rhinos. Larger open wounds would attribute to more protein loss and consumption than small lacerations or single small calibre gunshot wounds. More severe injuries would also be associated with reduced food intake, affecting the nutritional status of these animals.

Fibrinogen is an acute phase protein, non-specific to the exact anatomical location or cause of tissue disturbance, which has been shown to increase in acute cases of infection and trauma in various species (79-82). This increase in fibrinogen levels also appears to be the case in the white rhino as there was a significant increase in the injured individuals.

Abnormally high serum urea levels can be due to pre-renal, renal and post-renal impairments. Our study showed significantly higher urea concentrations in injured compared to the healthy rhinos. Pre-renal causes of elevated urea levels are possibly the most applicable and most likely due to increased protein catabolism and decreased glomerular filtration rate due to reduced renal perfusion seen in circulatory failure in injured animals (83). Dehydration, circulatory shock due to haemorrhage, tissue necrosis, fever and starvation are the most likely contributing factors to the abnormally high urea levels seen in this study. These injured rhinos suffer haemorrhage, are often less ambulatory and exposed to extreme temperatures. Wounds are often septic, and many areas of necrosis are often seen. Some more critically injured animals are often pyrexic or in shock. Anorexia was often seen in late stages of trauma and days leading up to death in non-survivors.

Another factor that plays a role in non-renal increased urea concentrations without associated increased creatinine concentrations is gastrointestinal (GI) haemorrhage (84). Very little is

known about the causes and occurrence of GI ulceration and subsequent GI haemorrhage in the white rhino. Further investigations are needed to establish if GI ulcerations develop in traumatised animals and cause haemorrhage that results in elevated urea concentrations. Evaluating faeces for occult blood is a recommendation made by the authors of this study. This test is sometimes done in horses and cattle to non-invasively investigate gastric ulcers (85,86). Without accompanied urinalysis, it is however very difficult to accurately rule out possible renal functional impairment in some of these injured individuals. Post-renal causes of increased urea concentrations such as urine outflow obstruction or urinary tract rupture seem to be less likely but should also be considered by the attending veterinarian.

In humans and dogs, trauma is often associated with metabolic acidosis caused by decreased tissue perfusion and oxygen supply, which also causes an increase in lactate resulting in lactic acid accumulation in the tissues, subsequently lowering the pH of blood and tissues (71, 87, 88). Acidosis has been shown in humans to cause increased calcium loss via the kidneys due to decreased renal tubular reabsorption (89). Sepsis and severe tissue injury are common differentials for total hypocalcaemia, and the most likely cause of lower levels of both ionised and total calcium concentrations in the injured rhinos.

Hypomagnesaemia is primarily caused by either decreased gastrointestinal absorption or increased renal loss, however many factors might lead to these causes (90). Other causes in domestic species include redistribution and hypoalbuminaemia (91). In humans, hypomagnesaemia at the time of admission of acutely ill patients is associated with increased mortality rates (92). Hypophosphataemia and hypocalcaemia are co-associated with hypomagnesaemia in 29% and 22% of humans respectively (93). Although redistribution of magnesium is not well understood, it is often seen in cases of severe tissue trauma and sepsis in many species and therefore we theorise that this may be the cause of the lower magnesium concentrations seen in our injured rhino.

Calcium, phosphorus and magnesium levels should however best be examined in conjunction, due to the co-association between these minerals and the influences they might have on each other. Abnormal levels in either of these is likely linked to abnormal levels in some of the others, as shown above.

Both AST and CK levels showed significant elevations in the injured compared to the healthy control rhinos. These enzymes have many iso-enzymes present in a variety of tissues in the body. AST is found in highest concentrations in hepatocytes, skeletal and cardiac muscle cells while CK is highest in skeletal, cardiac and smooth muscle and is considered a muscle-specific leakage enzyme (94). When serum AST activity is elevated in conjunction with serum CK activity, the origin of the AST is most likely from muscle injury as opposed to hepatocyte injury. The rate of response (time to reach peak serum activity levels) of these two enzymes differ with CK being much faster (peaking at 6-12 hours after injury with a serum half-life of about two hours in the horse and declines to normal levels within 48 hours of a singular acute injury event) than AST (peaking between 24 and 36 hours after injury and decreasing with a half-life of as much as eight days in the horse) (94). When serum activity of these enzymes are elevated beyond any of the abovementioned time periods, it indicates ongoing muscle damage. It is therefore recommended to evaluate CK in conjunction with AST when muscle damage, and its progression or resolution over time, needs to be investigated. Although the half-life of AST for the white rhino has not been formally studied before, it is expected that it would be similar to that of the horse (up to eight days).

Another possible cause for elevated CK activity to consider in critically injured rhinos is muscle catabolism in cases where individuals are anorexic. This phenomenon has been described in the cat (94).

Considering the severe muscle damage often seen due to trauma or ischaemia in injured rhinos, the elevated CK and AST activity seen in this study was thus an expected finding.

Serum lactate, together with the anion gap is often evaluated in critically ill dogs and horses to evaluate response to treatment as well as to determine prognosis (46, 88, 95, 96). Lactate has been shown to be stable in fluoride/oxalate tubes collected from rhino (36). Cole *et al*. (36) reported a mean of 4.6 mmol/L, which falls outside of our study's 95% confidence interval of 5.21 – 7.83 mmol/L, while our study reported a mean of 6.5 mmol/L in fluoride/oxalate. Their range still however falls within our reference interval.

An unexpected finding in our study was that lactate levels were not significantly different between the groups. The reasons are unclear and considering the small sample size of our study and the lack of serial measurements and blood-gas and anion gap data, any theory would be merely speculation. Multiple studies on lactate in the horse did however show significant increase in lactate concentration seen after exercise (97-100). Considering that many rhinos perform various degrees of physical activity during even routine immobilization,

evaluating the cause of elevated lactate levels would thus be difficult. Further studies on lactate in this species, with specific focus on increased sample size is needed before it can be ruled out as a measurand of value in clinical evaluation and prognostication.

During any form of injury, normal physiological responses include changes in endogenous hormone production, especially increased levels of cortisol. In humans this leads to insulin resistance which in turn leads to hyperglycaemia (101). Elevated cortisol and glucose levels are common findings in human trauma and hospitalised patients (102,103). Hyperglycaemia is also an indicator of poor prognosis in human trauma and horse colic patients (46,104). The nature and severity of the painful injuries seen in rhinos that are victims of poaching events, as well as the environmental stressors related to these attacks, such as being shot and chased and separated from their herds and young, leave no surprise that cortisol was significantly elevated in the injured individuals. Similarly it is not surprising that glucose was also elevated in these individuals.

Insufficient numbers of injured rhinos, especially non-survivors, in this study was the reason that the determination of prognostic indicators of survivability was not possible. The single most important reason for this was poor compliance from private veterinarians whom this study relied on to send samples to the OVAH laboratory. Additionally, from those that did comply with the sample requests, many samples were improperly handled during collection or transport that these samples could not be used. The wide geographical spread of rhino populations throughout South Africa added to this problem.

When looking at outcomes and comparing survivors with non-survivors, only magnesium levels were statistically significantly lower in non-survivors. In the horse, hypomagnesaemia has been shown to have no link with mortality, and horses with hypomagnesaemia even showed better survival rates in cases of colic (105). Various studies in multiple species concur that a holistic approach, looking at total body homeostasis, combining physical examination findings with laboratory findings, and serial measurements over time is the only way to accurately determine prognostic indicators and predict case outcome accurately (46, 95, 106). It is however important to emphasise that the number of non-survivors in our study was very low, and therefore it may be that other measurands could be different between survivors and non-survivors. Additionally, large sample sizes, especially including many more non-surviving individuals, are needed to draw any conclusions on prognostication (107).

In wild rhino it is very difficult to do serial measurements since repeated immobilisation is needed in order get samples and this may also have detrimental effects on an already compromised animal. Severe hypoxia due to opioid induced respiratory depression, pulmonary oedema, tachycardia, muscle rigidity and limb trembling are among the potentially dangerous side effects of immobilisation seen in healthy individuals (13, 108-113). These effects could potentiate the pathophysiology of a compromised severely injured animal and thus makes routine monitoring of injured rhinos a risky endeavour. The challenge to practitioners is that good clinical monitoring needs to be balanced with holistic patient wellbeing over the long term. A concerted effort between private practitioners and researchers should be made in order to obtain blood samples and other critical data from injured rhinos in order to enlarge the sample size of this study in order to amplify the findings of this study and allow for prognostication.

4.3 Recommendations:

Based on the findings from this study, the following recommendations can be made:

- Practitioners attending to injured rhinos should prioritise taking blood samples with each visit.
- Ensure proper sample handling during collection as well as during transport to the laboratory.
- Proper recording of immobilisation protocols as well as all physical examination findings are essential.
- In addition to a proper physical clinical examination, including the necessary diagnostics like imaging, running a CBC and measuring CK and AST together with calcium, phosphate and magnesium would be relevant.
- Urea levels should also be monitored.
- Perform post-mortem examinations to determine exact cause of death with all non-survivors.

Chapter 5: Conclusion

This study has various scientific as well as practical implications. We created reference intervals that are both species and analyser specific. Considering the limitations in interpretation as discussed, results from injured and otherwise ill or compromised rhinos can be compared with these reference intervals to detect abnormalities. *Theileria sp*. were seen in some individuals and its clinical significance needs further investigation in the white rhino, especially those that are injured. Similarly, the relevance of the high incidence of Heinz bodies needs further investigation in this species. Comparisons between non-injured and injured as well as between survivors and non-survivors highlighted some key clinical pathology measurands that are of importance in evaluating individual rhino patients. AST and CK should be evaluated together and show promise in evaluating progress of recovery in injured rhinos. Fibrinogen was shown, as in other species, to be a good indicator of acute trauma or infection. Abnormally high serum urea concentrations were seen in injured rhinos, and correcting this should be one of the focus points for the practitioner. Rectifying possible acidosis and fluid and electrolyte imbalances will aid to restore homeostasis in the patient. Its cause needs further investigation and a possible starting point is to see if GI ulceration commonly occurs during trauma, and if it leads to GI haemorrhage in the rhino. Investigations should ideally include a faecal occult blood test. Evaluation of calcium, phosphate and particularly magnesium showed significantly lower levels in injured rhinos, and should be included in the monitoring profiles that practitioners use to evaluate patients. Serial measurements of these measurands over the course of treatment in survivors is essential to monitor progress and could possibly help predict outcomes.

Due to the small sample size we were not able to determine if any of the measurands could be used to predict survival, so additional data and further research is required on more animals to help with prognostication. Without compliance from private practitioners, by collecting samples from every injured rhino they treat, future research will not be possible. The importance of lactate, particularly as a prognostic indicator in the white rhino, needs further evaluation with larger sample sizes before being ruled out. For adequate treatment and management of patients, a holistic approach to cases of injured rhinos is required which includes taking into account data from all possible observational and diagnostic modalities available in conjunction with the significant measurands in this study. The development of better treatment protocols, and the advancement of diagnostic equipment and capabilities is also required. Our results not only provide more reference information on blood measurands

but also indicate which of these are key to assess when animals are injured. Furthermore these findings highlight which organ systems are affected the most by injury, which is important to optimise treatment and may be useful to determine prognostication in the future.

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Appendix A

RHINO PROGNOSTIC INDICATOR STUDY - PRIMARY DATA COLLECTION SHEET									
Dr Jacques du Preez -0724023126	CONTROL ☐			POACHED ☐		OTHERWISE INJURED ☐			
Date:		Recorded by:				Owner + Vet:			
Animal Info									
Species: WR BR		Sex: M F			Age(E / K):				
Weight: (estimated)			Body Condition Score, out of 5:				Horns:		
Name &/ Nr:			Body Microchip Nr:				Habitus:		
Repro Status if *Male*: a)Territorial			b)Submissive		c)Immature		d) Unknown		
Repro Status if *Female*: a)Pregnant (S / K)			b)Open (S / K)		c)Calf at foot		d) Immature		
Measurements (cm and kg)									
FR FOOT:	Circumference:		**HINDFOOT:**		Circumference:				
	Length:	Width:			Length:		Width:		
Mass (only if measured, no estimations):			Actual Weight:		In Crate:		Crate:		
History									
1. Previous medical history/veterinary attendance:									
2. Poaching method:	a) Darted		b) Shot (type of weapon if known?):						
	c) Unknown:				d)Other:				
3. Interventions prior to veterinary assistance:									
*4. Interval since poaching/injury to veterinary attendance in hours: score: 1 2 3 4									
---------comments:									
5. Observations prior to immobilisation/from a distance: (appetite, locomotion, behavior/habitus)									
(normal/abnormal)									
Diagnosis, Treatment and Drugs (Immobilisation and Treatment)									
Drugs - name and amounts - for *immobilisation*:									
Helicopter Ground			Quality:				Total time:		
Specific diagnosis, if applicable:									
Drugs -name, route and amounts - used for *treatment*:									
Supportive therapy (oxygen or fluids) [rate/type + volume]:									
*Classification of Injuries: Score out of 25:						Score out of 5:			
--------comments:									
Details of treatment itself:									
Monitoring and Data Collection									
Serum x2:	EDTA:		Heparin x2:		Fluoride:		Citrate:		Faeces:
Lactate: Time:									
PL BL Measurement:									
Time:									
Rectal Temperature (°C):									
Heart Rate + Pulse Quality:									
MM/CRT:									
Resp. Rate + R. Effort (n,s,i):									
Pulse ox:									
Additional Comments/Information/Remarks:									