UNIVERSITY OF PRETORIA
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Ocular biometry and pathology in captive and free-ranging southern white rhinoceros *(Ceratotherium simum simum)* and south-central black rhinoceros *(Diceros bicornus minor)* in South Africa.

Final dissertation prepared in partial fulfilment of requirement for the MMedVet (Ophthal) degree.

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DECLARATION OF ORIGINALITY

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DEDICATION

I dedicate this thesis to the countless individuals, groups, organisations, private reserves, national and provincial reserves, wardens, and security personnel who, despite great adversity and considerable personal risk, are doing everything in their power to ensure the survival of these animals for future generations.

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LIST OF ABBREVIATIONS

ACD	Anterior chamber depth
AGL	Axial globe length
CI	Confidence interval
CITES	Convention of International Trade in Endangered Species of Wild Fauna and Flora
CLT	Crystalline lens thickness
D	Dioptre
IOL	Intraocular lens
IOP	Intraocular pressure
IUCN	International Union for Conservation of Nature
kg	Kilogram
km	Kilometre
m	Meter
mm	Millimetre
mmHg	Millimetres mercury
mm/min	Millimetres per minute
OD	Right eye, oculus dextra
ONH	Optic nerve head
OS	Left eye, oculus sinistra
OU	Both eyes, oculus uterque
PACD	Postoperative anterior chamber depth
PPM	Persistent pupillary membrane
PSD	Posterior segment depth
PTF	Precorneal tear film
SAVC	South African Veterinary Council
SD	Standard deviation
STT	Schirmer tear test

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ABSTRACT

Title:

Ocular biometry and pathology in captive and free-ranging southern white rhinoceros *(Ceratotherium simum simum)* and south-central black rhinoceros *(Diceros bicornus minor)* in South Africa.

Background:

The available literature describing rhinoceros' ocular abnormalities is limited. This may stem from the rarity of the animals and limited baseline ocular data available. The purpose of the project is to add to current knowledge regarding the normal ocular population parameters and prevalence of ocular pathology in rhinoceroses.

Methods:

Seventy-three immobilised rhinos underwent ophthalmic examination while immobilised for general veterinary care and procedures. The ophthalmic examinations were performed by the author of the study, a registered veterinarian with the South African Veterinary Council (SAVC), and by a registered SAVC specialist veterinary ophthalmologist, when he was available. The ophthalmic examination included the Schirmer Tear Test (STT), intraocular pressure (IOP), slitlamp biomicroscopy, fluorescein staining, keratometry and ocular ultrasonography and biometry. Exploratory data analysis was performed to establish the baseline parameters with binomial exact methods used to establish 95% confidence intervals for the estimate of the means of normal ocular parameters.

Results:

Seventy-three animals were examined, 68 were white rhino and 5 were black. Twenty-four were male and 49 were female. Mean STT OD: 19.09 mm/min (95%CI: 17.48 – 20.69); mean STT OS: 17.64 mm/min (95% CI: 16.14 – 19.14); mean IOP OD: 41.12 mmHg (95% CI: 36.74 – 45.51); mean IOP OS: 42.66 mmHg (95% CI: 38.52 – 46.81). The most common ocular abnormalities were keratitis (23 animals, 31.51%), corneal scar (12 animals, 16.44%), cataract (11 animals, 15.07%), corneal ulcer (7 animals, 9.59%), pigmentary keratitis (3 animals, 4.11%), corneal foreign body, posterior synechiae and persistent pupillary membrane was present in two animals each (2.74%), follicular conjunctivitis (1 animal, 1.37%); 6 right eyes were fluorescein positive (8.2%) and 3 left eyes were fluorescein positive (4.1%). Mean AGL OD: 26.2 mm (95% CI: 2.57 – 2.66); mean AGL OS: 26.0 mm m (95% CI: 2.55 – 2.65); mean ACD OD: 2.7 mm (95% CI: 0.25 – 0.28); OS: 2.7 mm (95% CI: 0.25 – 0.28 OS); mean CLT OD: 6.5 mm (95% CI: 0.64 – 0.66); mean CLT OS: 6.4 mm (95% CI: 0.63 – 0.66); mean PSD OD: 16.3 mm (95% CI: 1.6 – 1.66); mean PSD OS: 1.62 (95% CI: 1.6 – 1.65).

Conclusion:

The findings regarding normal biometry will aid in future examinations of the species. The prevalence of ocular disease is high and has seemingly little impact on their natural life.

Chapter 1

INTRODUCTION

Rhinoceroses are peculiar mammals that, in some way, resemble prehistoric, dinosaur-like animals that belong in a far away, ancient time. They are some of the most primitive of the world's large mammals (Radcliffe and Morkel, 2014). They inspired a beautiful and amusing tale by Rudyard Kipling, where he relates how the rhinoceros got his wrinkly, folded skin (Kipling, 2016). Three living families are included in the order Perissodactyla: the rhinoceroses, equids, and tapirs. All species included in this order bear weight on one (equids) or three (rhinoceroses and tapirs) digits, the sagittal plane of symmetry of the distal limb transects the third phalanx (Montiani-Ferreira et al., 2022; Radcliffe et al., 2014). Rhinoceroses are robust, large, odd toed ungulates and are part of the family *Rhinocerotidae* (Radcliffe and Morkel, 2014). The five extant species of rhinoceros include: the white rhinoceros *(Ceratotherium simum)*, the black rhinoceros *(Diceros bicornus)*, the Sumatran (or Asian two-horned) rhinoceros *(Dicerorhinus sumatrensis)*, the Indian (or greater one-horned) rhinoceros *(Rhinoceros unicornis)*, and the Javan (or lesser one-horned) rhinoceros *(Rhinoceros sondaicus)* (Skinner and Chimimba, 2005). Of the 5 extant species, 4 are critically endangered due to poaching and habitat loss and their distribution is limited to Africa and Asia (Radcliffe and Morkel, 2014). The two rhinoceros species occurring in the Southern-African subregion are the white and black rhinoceros, they arose from a common ancestor and the fossil record showed they occurred throughout Africa some four to three million years ago (Radcliffe and Morkel, 2014; Skinner and Chimimba, 2005).

The origin of the colloquial nomenclature of "white" and "black" rhinoceros is somewhat obscure with no clear or obvious origin (Rookmaaker, 2003). It was suggested by *Owen-Smith (1973)* that the name is related to the skin colour obtained when the white rhino wallows and rolls in calcareous soil.

The white and black rhinoceroses are threatened by severe poaching and habitat loss with the black rhinoceros appearing on the International Union for Conservation of Nature red list as critically endangered (IUCN, 2020a) and the white rhinoceros as near threatened (IUCN, 2020b). By the end of the 19th century, the once wide-spread white rhinoceros was on the brink of extinction with a small population of 20-50 animals in KwaZulu-Natal, South Africa, being the only remaining animals (IUCN, 2020b; Skinner and Chimimba, 2005). By the end of 2017, their numbers had decreased from an estimated 21,316 in 2012 to between 17,212 - 18,915. Five countries account for 99.3% of the remaining population, with South Africa conserving 18,933 individuals (~86.5%) by end 2012. The current population trend for white rhinoceros is declining (IUCN, 2020b).The most numerous of the world's rhino species by the end of the 20th century was the black rhinoceros. During the 1960's, an estimated 100 000 animals only, remained (IUCN, 2020a). The black rhino was saved from extinction by the establishment of the Hhluluwe and Imfolozi game reserves in 1895 and Mkhuze Game Reseve in 1912 (Skinner and Chimimba, 2005). It is a sad reality that their numbers further declined by 98% between 1960 - 1995 due to poaching and habitat loss. Their population numbers stayed stable and increased between 1992 - 1997 in South Africa and Namibia only. Black rhinoceros population numbers increased steadily and by end 2018 and estimated 5630 individuals existed and the current population trend is increasing (IUCN, 2020a).

Species	White rhino (WR) *Ceratotherium simum*				Black Rhino (BR) *Diceros bicornis*					Total
Subspecies/ Management Cluster	*C.s.cottoni (Northern)*	*C.s.simum (Southern)*	TotalWR	Trend	*D.b.bicornus (South-western)*	*D.b.michaeli (Eastern)*	*D.b.minor (South-eastern)*	TotalBR	Trend	*Both species*
Botswana		452	452	*Up*			50	50	*Up*	502
*(Cote d'Ivoire)***		1	1							1
Kenya	3	510	513	*Up*		745		745	*Up*	1,258
Malawi							28	28	*Up*	28
Mozambique		29	29				1	1		30
Namibia		975	975	*Up*	1,857			1,857	*Up*	2,832
Rwanda						19		19	*Up (New)*	19
*(Senegal)***		3	3							3
South Africa		15,625	15,625	*Down*	331	83	1,632	2,046	*Up*	17,671
eSwatini		66	66	*Down*			21	21	*Up*	87
Tanzania						155	5	160	*Up*	160
Uganda		22	22	*Up*						22
Zambia		14	14	*Up*			48	48	*Up*	62
Zimbabwe		367	367	*Up*			520	520	*Up*	887
End 2017 total	***3***	***18,064***	***18,067***	*Down*	***2,188***	***1,002***	***2,305***	***2,305***	*Up*	***23,562***
End 2015 total*	*3*	*20,053*	*20,056*	*Down*	*2,212*	*887*	*2,115*	*5,214*	*Up*	*25,27*
End 2012 total*	*4*	*21,316*	*21,32*	*20,165 in 2010*	*1,968*	*799*	*2,078*	*4,845*	*4,880 in 2010*	*26,165*

Table 1. Estimated numbers of White and Black Rhino by species and subspecies/genetic management cluster and by country as of the end of 2017, with marginally revised continental totals for end of 2012 and 2015. (From Emslie et al. 2019 and based on AfRSG data in collaboration with Range States)(Emslie et al., 2019; IUCN, 2020b). AfRSG African Rhino Specialist Group.

Chapter 2

LITERATURE REVIEW

Traditionally, rhinoceroses have been considered to be myopic (near sighted) with relatively poor eyesight (Howland et al., 1993; Pettigrew and Manger, 2008). However, streak retinoscopy and neutralising infrared video retinoscopy of three white rhinoceroses showed them to be, in fact, mildly hyperopic (Howland et al., 1993). *Johnson et al (1901)*, during his comparative anatomical examination of multiple mammalian specie eyes, performed refraction on all the animals he studied, with and without mydriatics, whenever possible. He found that, with a few notable exceptions, all Mammalia to possess hyperopic vision (Johnson, 1901). *Banks et al (2015)* evaluated pupil shape as a function of the ecological niche (foraging mode and time of day activity) exploited by terrestrial species. Rhinoceroses have a rounded pupil shape, and this is typically associated with cathemeral forage patterns in the absence of significant predation risk (Banks et al., 2015; Johnson, 1901). Retinal ganglion cell density in the visual streak for both the white- and black rhinoceros have a peculiar concentric concentration of ganglion cells in the temporal and nasal regions, creating an area centralis temporally and nasally. The visual streak lies dorsal to the optic nerve in the white rhinoceros and the optic nerve lies within the visual streak in the black rhinoceros (Coimbra and Manger, 2017; Pettigrew and Manger, 2008). The retinal ganglion cell density in the black rhinoceros suggests that their visual resolution is comparable to that of the rabbit and exceeds resolution seen in various mammals, including rats, seals, and dolphins, and about half the resolution seen in the domestic cat. The resolution of black rhinoceros vision is sufficient to allow detection of small leaves at relevant foraging distances (Pettigrew and Manger, 2008). The spatial resolution for both black and white rhinoceros was calculated to be 6-7 cycles/degree, compared to the 60 cycles/degree in humans. The conclusion by the authors of these studies was that the spatial topography of retinal ganglion cells allows the rhinoceroses to observe predators and conspecifics from in front and behind them while sampling the horizon during grazing in their preferred habitats. The authors also postulate that the distribution of retinal ganglion cells compensate for their reduced head and neck mobility which allows improved observation of their immediate surroundings (Coimbra and Manger, 2017; Pettigrew and Manger, 2008). The function of the tapetum lucidum is to provide retinal cells with an additional opportunity of photon catch, thus enhancing visual sensitivity during scotopic conditions (Ollivier et al., 2004). The tapetum is found among all perissodactyls (tapirs and equids) but not the greater one-horned rhinoceros. Ophthalmoscopic examination of a greater one horned rhinoceros showed a dull, uniform brown fundus colour which is consistent with the absence of a tapetum lucidum (Johnson, 1901). *Coimbra and Manger (2017)* found a similar appearing fundus during their examination of the white rhinoceros female eyes and noted that no vascular endothelium was observed in the specimen they examined (Coimbra and Manger, 2017). Considering these findings, it is suggestive that the rhinoceros has a paurangiotic retina where blood vessels are limited to the retina immediately adjacent to the optic nerve head (Allbaugh et al., 2016; Coimbra and Manger, 2017; Johnson, 1901).

The IOP and tear production values were determined for 11 white rhinoceroses by *Ofri et al., (2002)*. The mean intra-ocular pressure (IOP) measured in this study, using applanation tonometry (Tono-Pen XL applanation tonometer) was 32.1 ± 10.4 mmHg and tear flow was 17.6 ± 3.1 mm/min. The mean

IOP values are the highest recorded for any terrestrial species. Immobilisation of the animals in this study was achieved by using a combination of etorphine hydrochloride, butorphanol and detomidine (Ofri et al., 2002).

The tear production, IOP and ocular biometry was determined for five captive greater one-horned rhinoceroses *(Rhinoceros unicornis).* The mean Schirmer Tear Test 1 value was 18.2 ± 3.49 mm/min. The IOP was estimated using applanation tonometry (Tono-Pen applanation tonometer) and the mean value obtained was 32.1 ± 6.62 mmHg. Ocular biometry measurements were as follows: axial globe length 26.1 ± 1.1mm; corneal thickness 1.3 ± 0.1mm; anterior segment (chamber) depth 2.8 ± 0.6 mm; crystalline lens thickness 7.0 ± 1.1 mm and posterior segment depth 14.6 ± 1.3 mm. Immobilisation of the animals in this study was achieved by using a combination of detomidine and butorphanol tartrate (Bapodra and Wolfe, 2014).

2.1 Ophthalmic examination

Examination of the eye utilising various modalities is the only reliable means of assessing vision and possible pathology that may or may not be present. The following procedures, with the exception of keratometry and ocular ultrasonography and biometry, are performed during the routine veterinary ophthalmic examination for veterinary patients (Featherstone and Heinrich, 2021):

2.1.1 Schirmer Tear Test
2.1.2 Tonometry
2.1.3 Keratometry
2.1.4 Slit Lamp Biomicroscopy
2.1.5 Fluorescein Dye Test
2.1.6 Ocular Ultrasonography and Biometry

2.1.1 Schirmer Tear Test (STT)

The tear film is classically described as a three-layered liquid film consisting of an outer lipid layer, middle aqueous layer and an inner mucous layer (Davidson and Kuonen, 2004; Faghihi and Rajaei, 2023). The aqueous and lipid layer exist as an muco-aqueous phase with no clear boundary between the layers (Faghihi and Rajaei, 2023; Iwashita et al., 2023). The Schirmer Tear Test is the most used diagnostic test used to assess the aqueous tear production in dogs (Iwashita et al., 2023). The purpose of the STT is to quantify the production of the aqueous component of the tear film for the individual animal and remains the standard diagnostic test to quantify the aqueous tear production in veterinary ophthalmology. This test should be performed at the beginning of the ophthalmic examination to minimise reflex tearing (Featherstone and Heinrich, 2021; Gilger and Stoppini, 2016; Maggs, 2018). Basal and reflex tearing, the STT 1, is measured without using topical anaesthesia (Gilger and Stoppini, 2016). Tear absorption is non-linear and lacrimation kinetics describes a rapid, initial tear absorption that reflects the lacrimal lake volume, followed by a slower, steady state uptake that reflects tear production (Iwashita et al., 2023). The seven major functions of the tear film can be summarised as follows: maintaining a smooth corneal surface for proper light refraction; lubricating the eyelids; lubricating the conjunctiva and cornea; supplying the cornea with nutrients and removal of metabolic

by-products from the corneal surface; providing white blood cells with access to the cornea and conjunctiva; removing foreign materials from the corneal surface and conjunctiva; and ocular surface defence via specific and nonspecific antibacterial substances (Davidson and Kuonen, 2004; Hartley et al., 2006; Ofri et al., 2002).

A standardised strip of Whatman no. 41 filter paper has a notch and is impregnated with blue dye at the 5mm reading scale mark. The strip is folded at the notch, while still in the protective packaging to prevent contamination with oils from the examiner's hands and placed in the ventral fornix of the conjunctiva and the eyelid is then held closed. The strip dimensions are 5 x 35mm and the measuring scale is denoted in mm, the result is recorded in mm/min. It is left in place for 1 minute and read immediately. The effect of age, season, gender, environment, sex, time of day and placement of the STT strip have been described for horses and ponies and a wide variability in the STT 1 was found in normal horses and ponies (Beech et al., 2003). Of the above listed variables, the only variable within the examiner's control was placement of the STT strip. Care was taken to ensure that the strip was in contact with the corneal epithelium at all times and was the first diagnostic test performed in all research subjects.

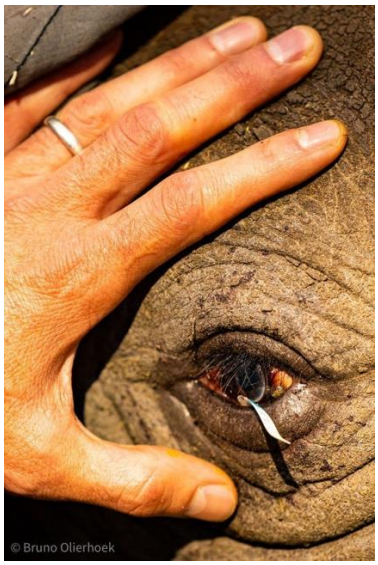


Figure 1. Measuring STT (credit, Bruno Olierhoek).

2.1.2 Tonometry

Tonometry is the measurement or estimation of intraocular pressure (IOP) and can be performed using either direct tonometry (manometry) or indirect tonometry (digital and instrumental tonometry) (Featherstone and Heinrich, 2021; Maggs, 2018). Atraumatic, accurate and repeatable estimates of IOP (in normal and diseased eyes) requiring minimal restraint with ease of use are all characteristics of the ideal tonometer. Accuracy across a wide species range with varying ocular anatomy is also required in the field of veterinary ophthalmology (Knollinger et al., 2005).

Direct tonometry via manometry, is invasive and not ethically justifiable in the clinical setting, even though it is the gold standard and most accurate method of determining IOP (Featherstone and Heinrich, 2021; Maggs, 2018; Mustikka et al., 2020).

Digital tonometry is the estimation of IOP via digital palpation. It involves placing the index finger on the eye over the closed eyelid and gently applying pressure to the globe, preferably both eyes simultaneously, to estimate how hard the eye feels. The technique is crude and will mostly differentiate between very soft and very hard eyes. As such, it is not very sensitive and will not provide accurate measurements of IOP (Featherstone and Heinrich, 2021; Maggs, 2018).

Instrumental tonometry implies estimation of IOP through measuring corneal tension and is the technique of choice in clinical veterinary ophthalmology. It is a non-invasive, quick, and straightforward procedure that is performed with minimal patient discomfort. Indentation tonometry, applanation tonometry and rebound tonometry are all examples of instrumental tonometry (Featherstone and Heinrich, 2021; Maggs, 2018).

The indentation, or Schiotz tonometer, was developed and described in 1905. In this method, the cornea is anaesthetised, and a weighted plunger applies a standard force to the cornea. The amount of corneal indentation applied by a given weight is measured and the IOP is inversely proportional to the degree of corneal indentation. The instrument relies on gravity and must be placed vertically on the central horizontal cornea, this implies that the animal must be restrained in a manner that allows the instrument to be oriented vertically. Correct application of the instrument on veterinary patients, especially species with laterally positioned globes, creates some difficulty and could lead to inaccurate readings (Featherstone and Heinrich, 2021; Maggs, 2018).

Applanation tonometry is based on the principle of Goldmann's Imbert-Fick law:

> "*The pressure in a sphere filled with liquid and surrounded by an infinitely thin membrane is measured by the counter-pressure which just flattens the membrane"*.

The IOP is estimated by measuring the force required to flatten, or applanate, a known area of cornea where pressure = force/area. Topical anaesthesia is required for use of the applanation tonometer and readings from the central two-thirds of the cornea are most accurate (Featherstone and Heinrich, 2021; Maggs, 2018).

Rebound tonometry estimates the IOP by measuring the rebound characteristics of the cornea (Featherstone and Heinrich, 2021; Knollinger et al., 2005; Kontiola, 2000; Maggs, 2018). This rebound, or, deceleration, of a probe that is electromagnetically propelled onto the cornea from a set distance is measured and the IOP is estimated using this rebound characteristic (Knollinger et al., 2005; Kontiola, 1997, 1997; Maggs, 2018; Wang et al., 2005). Slower deceleration and shorter return time of the probe is present in eyes with higher IOP compared to eyes with a lower IOP (Featherstone and Heinrich, 2021; Maggs, 2018). The mean IOP estimate is obtained by measuring and automatic averaging of six consecutive readings. The procedure is non-invasive and quick with a very small footprint and does not require local anaesthesia for use of the tonometer (Knollinger et al., 2005; Kontiola, 2000). It has been demonstrated in the dog, cat and horse that the rebound tonometer has a strong linear correlation to direct manometry (Knollinger et al., 2005). Horizontal orientation of the rebound tonometer probe is

required for its proper function, and this is possible in standing animals or animals in sternal recumbency (Knollinger et al., 2005). This was an advantage in this study considering the research subjects were, in most cases, in sternal recumbency. The iCare TonoVet rebound tonometer was used, the researcher is familiar with the device, and it was freely available for use.

Numerous factors may influence IOP readings, and some factors are beyond the control of the examiner, e.g., age and gender of the animal and season. Other factors that may influence tonometric readings are improper restraint and head positioning, sedation, general anaesthesia, regional nerve blocks and type of tonometer used (Featherstone and Heinrich, 2021). Further, the rigidity, curvature and thickness of the rhinoceros' cornea may contribute to the increased IOP estimates recorded in this species (Ofri et al., 2002). Extraocular muscle tone, retractor bulbi contraction (leads to retraction of the globe into the orbit) and closure of eyelids will affect IOP. Further, blood pressure plays an important role in aqueous humor formation. Chemical restraint may affect any, or all, of these factors (Ofri et al., 1998a).

Figure 2. Estimating IOP with TonoVet rebound tonometer.

2.1.3 Keratometry

Most of the refractive power of the eye is created by the anterior cornea-air interface and keratometry is the determination of the curvature of this anterior corneal surface. This measurement is expressed in dioptres (D) or in mm of radius of curvature and involves measuring the steepest and flattest meridian (Han et al., 2022; Ramakrishnan and Naik, 2014). In humans, myopia is associated with longer axial globe lengths (AGL) (Han et al., 2022). The corneal curvature has a profound impact on the optical and refractive properties of the eye (Ramakrishnan and Naik, 2014). Auto-refract keratometry is a non-invasive procedure which allows obtaining these measurements rapidly (Kawasaki et al., 2020). The instrument shines a well-lit target, or mire, on the corneal surface, which acts as a convex mirror. This produces a virtual image of the target, and the corneal radius of curvature is predicted by the instrument using this image. The mean corneal radius of curvature (mm) and mean corneal refractive power (dioptres, D) is obtained by averaging two values, R1 and R2 for mm and K1 or K2 for dioptres (Kawasaki et al., 2020; McMullen and Gilger, 2006). The maximum radius of curvature ("flattest" curve)

and the lowest power meridian is represented by R1 or K1. The minimum radius of curvature ("steepest" curve) and highest power meridian is represented by R2 or K2. A major limitation of performing automatic hand-held keratometry on veterinary species is possible measurement errors. Examples of such errors are instability of operator hand holding the device or poor vision fixation due to animal movement. A reliable measurement for the *Kawasaki et al (2020)* study was defined as follows: both right and left eyes were measured successfully, and the difference in the R1R2avg between the right and left eyes was less than 4.5 per cent. Inter-operator variability was further reduced by using a single, trained operator for obtaining measurements (Kawasaki et al., 2020).

Predicting intraocular lens (IOL) dioptric strength in humans is performed through using one of several available theoretical formulas, these formulae are based on optical models of the eye (Butcher and O'Brien, 1991; McMullen and Gilger, 2006). Specific properties of the eye, which influence its optical and refractive state, are measured and entered into the formulas. Corneal curvature, estimated postoperative anterior chamber depth (PACD), and axial length of the eye are the required measurements to calculate IOL strength (Binkhorst, 1972; Retzlaff, 1980). The PACD is an estimation of the position the IOL will occupy after surgery and is a critical measurement to estimate IOL strength accurately. The position of the post-operative IOL is accurately known in human subjects given analysis of the vast amount of data available for the IOL position (McMullen and Gilger, 2006). *McMullen et al (2006)* determined the ocular biometry and corneal curvature in 14 horses utilizing A- and B-mode ultrasonography to calculate the intraocular lens (IOL) power for horses undergoing cataract extraction surgery. In the McMullen and Gilger study, the PACD was calculated as the distance between the corneal epithelium and the centre of the lens, which is equal to the preoperative anterior chamber depth (ACD) plus half crystalline lens thickness (CLT) (PACD = ACD+1/2 CLT). Because the actual postoperative position of the IOL is unpredictable, IOL power was also calculated for 2mm anterior and 2mm posterior to the PACD measurement (McMullen and Gilger, 2006). Ten enucleated equine globes were used to measure the lens diameter to determine the appropriate-sized IOL for use in 6 enucleated equine globes and 4 equine eyes. The mean ratio of preoperative to postoperative ACD was 0.73 (McMullen et al., 2010). *Meister et al (2018)* calculated the IOL power from retinoscopy, keratometry and ultrasonographic biometry on 98 healthy equine eyes from 49 horses. The PACD value in this study was calculated as ACD/0.73 (Meister et al., 2018). The Binkhorst and Retzlaff formulas were used to determine the IOL power for the horses included in both the *McMullen* and *Meister* studies (Binkhorst, 1972; McMullen and Gilger, 2006; Meister et al., 2018; Retzlaff, 1980).

The Binkhorst formula is described as follows (Binkhorst, 1972):

$$Pe = 1.366\frac{4r - L}{(L - C)(4r - C)}$$

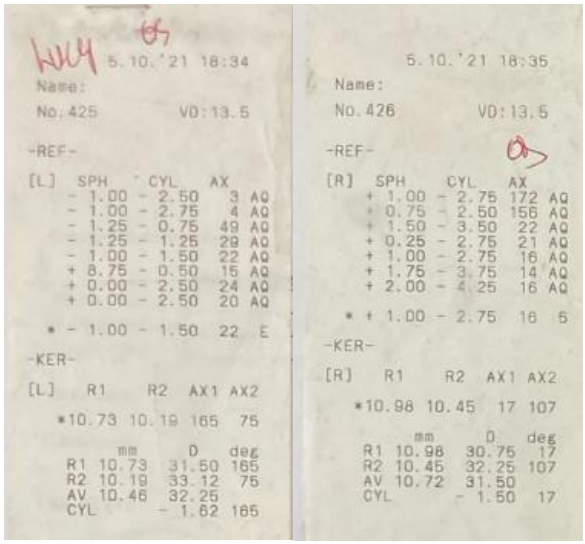
where: Pe = emmetropic IOL power (D); L = axial length of eye (mm); C = pseudophakic depth of the IOL/PACD; r = averaged corneal radius in mm.

The Retzlaff formula is described as follows (Retzlaff, 1980):

$$Pe = \frac{N}{L - C} - N \cdot \frac{K}{N - KC}$$

where: Pe = emmetropic IOL power (D); N = refractive index of aqueous and vitreous (1.336); K = corneal curvature in dioptres (D); C = PACD in m (assuming this to be the IOL location).

Figure 3. Performing keratometry in field conditions.



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	- 1.00	- 2.50	3	AQ
	- 1.00	- 2.75	4	AQ
	- 1.25	- 0.75	49	AQ
	- 1.25	- 1.25	29	AQ
	- 1.00	- 1.50	22	AQ
	+ 8.75	- 0.50	15	AQ
	+ 0.00	- 2.50	24	AQ
	+ 0.00	- 2.50	20	AQ
*	- 1.00	- 1.50	22	E

-KER-

[L]	R1	R2	AX1	AX2
	*10.73	10.19	165	75

	mm	D	deg
R1	10.73	31.50	165
R2	10.19	33.12	75
AV	10.46	32.25	
CYL		- 1.62	165

5.10.'21 18:35
Name:
No. 426 VD: 13.5

-REF-

[R]	SPH	CYL	AX	
	+ 1.00	- 2.75	172	AQ
	+ 0.75	- 2.50	156	AQ
	+ 1.50	- 3.50	22	AQ
	+ 0.25	- 2.75	21	AQ
	+ 1.00	- 2.75	16	AQ
	+ 1.75	- 3.75	14	AQ
	+ 2.00	- 4.25	16	AQ
*	+ 1.00	- 2.75	16	5

-KER-

[R]	R1	R2	AX1	AX2
	*10.98	10.45	17	107

	mm	D	deg
R1	10.98	30.75	17
R2	10.45	32.25	107
AV	10.72	31.50	
CYL		- 1.50	17

Figure 4. Keratometry data sheet.

2.1.4 Slit Lamp Biomicroscopy

The instrument of choice to examine the anterior ocular segment is the slit lamp biomicroscope. Three-dimensional visualisation of the adnexa, conjunctiva, sclera, cornea, anterior chamber, iris, lens and anterior vitreous is greatly improved through the magnification and the bright, focused light source provided by the instrument. The magnification offered by the hand-held Keeler PSL classic portable slit lamp is 10x-16x and the light beam options are a range of diffuse (round 12mm light beam), focused (square 1mm light beam) to slit (0.15, 0.5, 0.8 and 1.6 mm). The light beam is angled 20-45 degrees from the axis of the oculars at a focal distance of 7-10cm. Fine focus is achieved by moving very slightly either towards or away from the structure being examined. The light source can also be moved to different sides of the microscope to facilitate visualisation of structures. Serial, magnified examination

of the adnexa, surface- and intraocular structures, with varying forms of illumination as provided by the instrument, helps to create an overview of the ocular structures and aids in accurately identifying pathology, when present (Featherstone and Heinrich, 2021; Maggs, 2018).

Figure 5. Illustrating slitlamp biomicroscopy in field conditions, uncovered.

Figure 6. Illustrating slitlamp biomicroscopy under field conditions while covered.

2.1.5 Fluorescein Dye Test

Sodium fluorescein is an orange, weakly acidic, water soluble, highly lipophobic and hydrophilic dye. Its peak light absorption wavelength is 490nm (i.e., blue light) and when illuminated with blue light, it

emits green, fluorescent light with peak wavelength of 520nm. Fluorescence is most intense when the dye is dissolved in an alkaline environment (Featherstone and Heinrich, 2021). Strips of paper with fluorescein impregnated tips are applied to the bulbar conjunctiva and closing the eyelids distributes the dye across the cornea. Its lipophobic properties ensures it is not absorbed by intact lipid containing corneal and conjunctival epithelium. It will, however, adhere to exposed corneal and conjunctival stroma which makes it an excellent tool to identify corneal and conjunctival stromal ulcerations (Maggs, 2018). Fluorescein stain is primarily used to identify corneal epithelial ulcerations. It is also used to aid evaluations and diagnosis of descemetocele, full thickness corneal perforations (Seidel test) and patency of nasolacrimal ducts (Jones test) (Maggs, 2018).

2.1.6 Ocular Ultrasonography and Biometry

Ultrasonography is a non-invasive and easily accessible imaging modality that allows assessment of the anterior chamber, iris, lens, posterior segment, retina, and retro-bulbar space. Ultrasound frequencies used for ocular diagnostics range from 7.5 to 50MHz. Lower frequency ultrasound waves allow good tissue penetration and visualisation of deeper structures e.g., retro-bulbar area. High frequency (higher resolution) ultrasound waves penetrate tissue only a few millimetres and are ideal for imaging anterior ocular structures like the cornea and anterior chamber. The type of transducer, frequency, focal length, and beam aperture are all factors that influence resolution of the image. Typically, 10 - 15MHz, B-mode ultrasonography is most used in veterinary ophthalmology and can penetrate 60-40mm. Focal depth and resolution is not synonymous with penetration, nor is it a fixed given number. Rather, it depends on tissue characteristics, e.g., tissue density, backscatter and relative attenuation and transducer acoustic output. The eye is fluid filled and the tissue is hydrated, with low attenuation values and these characteristics favour penetration of sound waves (Bentley et al., 2021; Maggs, 2018).

Following electric energy application to the piezoelectric crystals, the crystals vibrate or “pulse” a soundwave into the tissue, which is followed by a pause. The returning soundwave, or echo, will then re-vibrate the piezoelectric crystals, and this produces a second electrical impulse which is then displayed as the ultrasound image. The image, or “real-time” display, is created by combining thousands of these pulse-echo combinations (Bentley et al., 2021; Gilger and Stoppini, 2016). The interface created at the junction of two media with differing acoustic impedance, creates this echo. If two opposing media have similar acoustic impedance no image will be created. Thus, the greater the acoustic impedance between two tissues is, the stronger the echo will be (Gilger and Stoppini, 2016). Strong echoes will occur when the soundwaves strike a smooth, flat interface perpendicularly and most, or all, of the wave energy is reflected/echoed back to the transducer. When the sound waves strike a surface at an angle, some of the wave energy is deflected away from the transducer and a weaker echo will result (Ledbetter and Van Hatten, 2017).

During A-scan ultrasonography, a graph-like, one dimensional, peaked pattern is created from returning echoes, which are plotted against time. The strength, or amplitude of the echo, is represented by the peak height and the time between peaks depicts distance, or tissue dimension (Bentley et al., 2021).

The B-scan, or brightness scan, produce a two-dimensional acoustic image, or slice, of the tissue and is a summation of multiple A-scans where each echo is represented as a dot. The strength, or amplitude of the echo, is represented by the brightness of the dot (Bentley et al., 2021).

Biometry is the measurement of intraocular and orbital structure dimensions. Biometric measurements of the globe and intra-ocular structures include the following: axial globe length (AGL), crystalline lens thickness (CLT), anterior chamber (ACD) depth and posterior segment depth (PSD) (Bentley et al., 2021; Maggs, 2018). For the purpose of this study, the biometry measurements were made as follows: AGL – anterior corneal epithelium to posterior sclera; ACD – posterior corneal endothelium to anterior lens capsule; CLT – anterior lens capsule to posterior lens capsule; PSD – posterior lens capsule to posterior sclera.

Figure 7. Illustrating ocular ultrasound and biometry under field conditions

2.2 Ocular abnormalities

The available literature describing rhinoceros' ocular abnormalities are limited. This may stem from the rarity of the animals and limited baseline ocular data available (Horowitz et al., 2016). Some of the reports describe management of corneal ulceration resulting from suspected trauma in greater one-horned rhinoceroses (Esson, 2015; Gandolf et al., 2000), one case of enucleation in a black rhinoceros following proptosis (Prole, 1965) and one case of conjunctival habronemiasis in a white rhinoceros female (Horowitz et al., 2016). There is a report describing an ocular disorder observed in a captive male and female Sumatran rhinoceros. The affected animals developed extensive ocular lesions which progressed to complete blindness in the female and partial blindness of the right eye, in the male. The aetiology of this ocular syndrome is unknown (Kretzschmar et al., 2009).

Eight rhinoceroses that underwent ophthalmological examination by Dr Antony Goodhead of Johannesburg Animal Eye Hospital had incidental ocular pathology discovered. Six of the eight animals examined had bilateral cataracts diagnosed and one animal had a unilateral cataract. Embryonic vascular abnormalities / posterior segment pathology was also present in half of the rhinoceroses in this case series. Four animals had a persistent hyaloid artery (PHA) of which three were associated with bilateral cataracts. Retinal detachment was also present in two animals diagnosed with PHA *(pers*

comm). Dr Goodhead performed bilateral enucleation on a young, orphaned rhino calf after she developed severe corneal ulcerations that lead to corneal perforations, following her translocation to a rhino orphanage facility. This calf survived and reached adult size and has subsequently produced a young calf of her own, despite her blindness. She is kept in a small, natural veld camp with other rhinos, and she is remarkably well adapted to living with her loss of vision *(pers comm).* A wild, 4-year-old, male black rhino was treated in a boma for severe leg wound and corneal pathology was observed while undergoing treatment for the leg wound. The eye examination revealed a central, yellow to beige appearing lesion within the corneal stroma with only moderate vascular keratitis associated with the lesion. Removal of the lesion, via lamellar keratectomy and followed by placement of a conjunctival pedicle graft in the resultant corneal defect, was pursued. Histopathology of the keratectomy sample revealed an intense distribution of dichotomous branching and septate hyphae. A sample for culture was not submitted and PCR of the paraffin embedded keratectomy sample failed to identify the pathogen. The eye was treated 4 times per day with topical fluconazole and enrofloxacin with a hand-held spray gun. The conjunctival pedicle graft was surgically resected 5 weeks after the initial surgery and the eye responded well to treatment with only minimal scarring present at time of last examination *(A Goodhead, pers comm)*.

There are no published records describing cataracts and posterior segment pathology in rhinoceroses to the authors knowledge.

Chapter 3

AIMS, OBJECTIVES AND BENEFITS

3.1 Research questions:

1) What are the population parameters for, STT and IOP and ocular biometry in immobilised rhinoceroses in South Africa?
2) What is the prevalence of ocular pathology in rhinoceroses in South Africa?

3.2 Aims and objectives of the study.

Aim

To describe the biometry of the rhinoceros' eye, establish mean STT and IOP values for immobilised rhinoceroses and to document ocular pathology for rhinoceroses.

Objectives

a) Description of the rhinoceros eye biometry and description of posterior segment pathology, if present.
b) Quantify the production of the aqueous component of the precorneal tear film in immobilised rhinoceroses.
c) Estimating the intraocular pressure (IOP) by indirect measurement for immobilised rhinoceroses.
d) Describing any ocular pathology present and explore potential univariate causal factors associated with pathology, based on subject demographics/signalment.

3.3 Benefits arising from the study.

The project will add to current knowledge regarding the normal ocular population parameters and prevalence of ocular pathology in rhinoceroses. The biometry and keratometry measurements may aid the calculation of the intraocular lens power, which may assist in future should cataract surgery and intraocular lens implantation ever be considered in the species.

Chapter 4
MATERIALS AND METHODS

4.1 Model system and justification

Establishing prevalence of pathology was undertaken using a cross-sectional observational study design requiring a sample size to estimate a binary outcome – pathology present or pathology absent. The sample size was established by:

$$n \geq \frac{z^2 \times N \times P_{exp} \times (1 - P_{exp})}{[(N-1) \times \epsilon^2 \times {P_{exp}}^2] + z^2 \times P_{exp} \times (1 - P_{exp})}$$

where;

Parameter	Description	Value used
z	reflects desired level of confidence	1.96 for 95% - i.e., $\alpha = 0.05$
N	population	Estimated at 17000 rhinoceroses in South Africa (IUCN, 2020b)
P_{exp}	Estimated population mean of pathology	0.05
ε	the maximum relative difference between the estimate and the unknown population value.	1 (in this case the confidence interval expected around the 5% expected prevalence will be between 0 and 10% which is 100% of the expected prevalence on either side – hence a ε value of 1)

Table 2. Parameters, description, and values used to determine sample size.

resulting in a sample size of 73 animals.

To estimate population parameters of continuous variables using simple random sampling, the sample size was calculated using the estimation established by Lemeshow and described by Stevenson (Levy and Lemeshow, 2008; Stevenson, 2021). Using data from publications by *Ofri et al., (2002)* and *Bapodra and Wolfe, (2014)*, the equation used to calculate the sample size to estimate a continuous outcome variable using simple random sampling is:

$$n \geq \frac{z^2 \times N \times {V_r}^2}{(z^2 \times {V_r}^2) + ((N-1) \times \varepsilon^2)}$$

where;

<table>
<tr><th>Parameter</th><th>Description</th><th>Value used</th></tr>
<tr><td>z</td><td>reflects desired level of confidence</td><td>1.96 for 95% - i.e., $\alpha = 0.05$</td></tr>
<tr><td>N</td><td>population</td><td>Estimated at 17000 rhinoceroses in South Africa (IUCN, 2020b)</td></tr>
<tr><td>V_r</td><td>the relative variance in population</td><td>Estimated from previously published work and defined by $\frac{\sigma^2}{\overline{X}^2}$</td></tr>
<tr><td>σ</td><td>standard deviation of continuous variable to be estimated</td><td><table><tr><th>Variable and reference</th><th>Value</th></tr><tr><td>IOP (Ofri et al., 2002)</td><td>10.4</td></tr><tr><td>IOP (Bapodra and Wolfe, 2014)</td><td>6.62</td></tr><tr><td>STT (Ofri et al., 2002)</td><td>3.1</td></tr><tr><td>STT (Bapodra and Wolfe, 2014)</td><td>3.49</td></tr><tr><td>Axial length (Bapodra and Wolfe, 2014)</td><td>0.11</td></tr><tr><td>Corneal thickness (Bapodra and Wolfe, 2014)</td><td>0.01</td></tr><tr><td>Anterior chamber depth (Bapodra and Wolfe, 2014)</td><td>0.06</td></tr><tr><td>Lens depth (Bapodra and Wolfe, 2014)</td><td>0.11</td></tr><tr><td>Posterior segment depth (Bapodra and Wolfe, 2014)</td><td>0.13</td></tr></table></td></tr>
<tr><td>$\overline{X}^2$</td><td>expected mean of the continuous variable to be estimated based on a sample size of 11 (Ofri et al., 2002) and 5 (Bapodra and Wolfe, 2014) animals in the selected studies</td><td><table><tr><th>Variable and reference</th><th>Value</th></tr><tr><td>IOP (Ofri et al., 2002)</td><td>32.1</td></tr><tr><td>IOP (Bapodra and Wolfe, 2014)</td><td>31.2</td></tr><tr><td>STT (Ofri et al., 2002)</td><td>17.6</td></tr><tr><td>STT (Bapodra and Wolfe, 2014)</td><td>18.2</td></tr><tr><td>Axial length (Bapodra and Wolfe, 2014)</td><td>2.61</td></tr><tr><td>Corneal thickness (Bapodra and Wolfe, 2014)</td><td>0.13</td></tr><tr><td>Anterior chamber depth (Bapodra and Wolfe, 2014)</td><td>0.28</td></tr><tr><td>Lens depth (Bapodra and Wolfe, 2014)</td><td>0.7</td></tr><tr><td>Posterior segment depth (Bapodra and Wolfe, 2014)</td><td>1.46</td></tr></table></td></tr>
<tr><td>ϵ</td><td>maximum relative difference between the estimate and the unknown population value – i.e., to yield an estimate that is within ε of the true population value?</td><td>0.05</td></tr>
</table>

Table 3. Parameters, description, and values used to estimate population parameters of continuous variables using simple random sampling.

Considering these variables, the sample size required to establish these population parameters would be:

Parameter	Based on	Sample size
IOP	Ofri et al., (2002)	**160**
IOP	Bapodra and Wolfe, (2014)	**69**
STT	Ofri et al., (2002)	**48**
STT	Bapodra and Wolfe, (2014)	**57**
Axial length	Bapodra and Wolfe, (2014)	**3**
Corneal thickness	Bapodra and Wolfe, (2014)	**10**
Anterior chamber depth	Bapodra and Wolfe, (2014)	**71**
Lens depth	Bapodra and Wolfe, (2014)	**38**
Posterior segment depth	Bapodra and Wolfe, (2014)	**13**

Table 4. Sample size calculation based on previous published data for rhino species.

An overall sample size of ~73 animals allowed establishment of population parameters and allows for an estimation of prevalence of pathology (at an estimated 5% incidence). The sample size of 160 animals in the *Ofri et al., (2002)* IOP study is due to the wide standard deviation that was estimated around the mean measurement in that trial. The authors raised concerns regarding the high IOP

estimates, although *Bapodra and Wolfe, (2014)* found similar estimates with narrower standard deviations.
Sampling 160 rhinoceroses is not logistically feasible and based on multiple parameters, 73 animals provide adequate estimates of both prevalence of pathology and population parameters.

Signalment characteristics included the following:

1) Age
2) Sex
3) Reproductive status (pregnant female, if known)

Demographic characteristics included the following:

1) Location in South Africa
2) Free ranging/wild vs captive
3) If captive, is animal housed in a boma or camps?
4) If captive in a boma, is animal housed singly or in a group?
5) Reason for capture (translocation, veterinary care)

4.2 Study Design

The study design is an observational, cross sectional ocular examination of rhinoceros eyes. It is a live animal model utilising 73 rhinoceroses.

4.3 Study population

Populations of captive or wild white and black rhinoceroses were examined at various locations in South Africa while fully immobilised for routine horn reduction and/or additional veterinary work, as required. Body size and horn length were used to determine age of rhinoceros (Hooijberg et al., 2020).

a) adult male (> 7 years)
b) adult female (> 7 years)
c) subadult male (3 - 7 years)
d) subadult female (3 - 7 years)

Basic ophthalmic examination of calves was performed when possible.

Identification of animals via ear notching, ear tags and/or implanted microchip will be a function of the facility where the animals will be examined. If an animal with no positive identification is immobilised, the identification strategy employed was recorded in the data capture sheet as required.

4.4 Staff and Facilities

All rhinoceroses were examined while under the care of an experienced wildlife veterinarian and under field conditions. To reduce risk of heat stress, immobilisations took place during the cooler, early morning and when possible, cooler months of the year, typically March to September. The following persons were involved with this research project:

1) Dr Paul Burger - ophthalmic examinations, data collection and documentation
2) Dr Antony Goodhead - Supervisor and ophthalmic examinations
3) Dr John Grewar – Co-supervisor, statistical analyses, and data evaluation
4) Wildlife veterinarians, wardens, and anti-poaching security staff at various locations

4.5 Study procedure

A complete ocular examination was performed by the study author [an MMedVet Ophthalmology resident and registered SAVC veterinarian] and, when available by, a SAVC registered specialist veterinary ophthalmologist. Examinations took place in field conditions and were determined by location of animals identified for immobilisation.

1) Immobilisation of all animals was performed by an experienced wildlife veterinarian using a remote drug delivery system. The drugs were delivered in the nuchal hump or gluteal area and the dosages were adapted to suit age, physical condition, and stress of animal. Drugs used for immobilising rhinoceros were determined by the attending wildlife veterinarian and included the following: etorphine hydrochloride (Captivon 98, Wildlife Pharmaceuticals Pty Ltd, White River, South Africa), azaperone (Stresnil, Elanco Animal Health, 9 Vervoer Street, Johannesburg, South Africa), medetomidine (Medetomidine, V-Tech, Corner Douglas and Old Pretoria Road, Midrand, South Africa), and hyaluronidase (Hyaluronidase, Kyron, 29 Barney Road, Benrose, Johannesburg, South Africa) (Haw et al., 2015, 2014; Miller et al., 2012). Intravenous administration of butorphanol (Dolorex, MSD (Pty) Ltd South Africa, Halfway House, Midrand) and oxygen insufflation was initiated, when possible, once the animal became recumbent (Haw et al., 2015, 2014). When the animals were safe to handle, the ground teams approached them, placed blindfolds, and positioned them in lateral or sternal recumbency, ensuring their heads were level and the lower nostril patent. Positive identification via microchip, ear notch or ear tag was performed.
2) Tear production was measured first using STT (Standardised STT, MSD (Pty) Ltd South Africa, Halfway House, Midrand) when possible and before any manipulation of the eye or adnexa occurred. This was to prevent reflex tearing and falsely elevated STT values.
3) Rebound tonometry was performed on both eyes using the iCare TonoVet rebound tonometer (Tonovet, Äyritie, Vantaa, Finland) when possible.
4) Keratometry using a hand-held, auto-refract keratometer (Nidek HandyRef-K, 34-14 Maehama, Hiroishi-cho, Gamagori, Japan) was obtained with animals in recumbency. The manufacturer's instructions were followed for obtaining of measurements. Multiple, automatic readings of the minor meridian (R1) and major meridian (R2) of the central cornea were obtained consecutively, when possible.
5) Slit Lamp Biomicroscopy examination of both globes was performed using the Keeler Classic Portable Slit Lamp Biomicroscope (Keeler Ltd, Winsor, UK). General examination for pathology of the globe, examining the eyelids, adnexa, cornea, anterior chamber, iris, lens, and posterior segment using the diffuse light beam, was performed. A focal slit beam was then used to evaluate the cornea, iris, lens and anterior chamber in more detail plus for evaluation for aqueous flare.

Considering the field conditions, the ocular examination took take place under a protective covering to minimise sunlight penetration during the examination.

6) Fluorescein stain (FLUO 900, Haag-Streit, Gartenstadtstrasse 10, Koeniz, Switzerland) was used in the next step of the ophthalmic evaluation. After application of the dye, the corneas were examined with a cobalt blue light and any pathology was recorded.
7) The ophthalmic examination concluded with ultrasound evaluation and biometry of the globe. Coupling gel was applied on the cornea to reduce near-field reverberation. A GE Logiq-e veterinary ultrasound machine (IMV Imaging, Blueberry Office Park, Apple Street, Honeydew, Johannesburg, South Africa) with a 12 – 13 MHz linear, B-mode transducer and 3-4 cm focal range was placed directly on the cornea in the horizontal plane (transverse image). The following measurements were recorded for both eyes to complete the biometry: axial globe length (AGL), anterior chamber depth (ACD), crystalline lens thickness (CLT), and posterior segment depth (PSD).
8) Following the ophthalmic examination, both globes were treated with an ocular lubricant (Artelac Advanced Lipids, Bausch & Lomb, Brunsbütteler Damm, Berlin) to reduce risk of desiccation of the corneal epithelium.
9) Recovery took place under direct supervision of the wildlife veterinarian. The effects of etorphine was reversed with naltrexone (Trexonil, Wildlife Pharmaceuticals Pty Ltd, White River, South Africa) administered in the auricular vein at 20-times the etorphine dose (Haw et al., 2015, 2014).

The primary investigator acknowledges that complete data set for each animal examined was not possible. Any deviations from a complete examination will be noted and analysis will reflect incomplete data.

4.6 Data Analysis and availability

General descriptive count and proportions were established for all categorical variables in the dataset. For numerical parameters and prevalence estimates of pathology: sample proportions and means with 95% confidence intervals were established – the latter using binomial exact methods. Comparisons between left and right eyes, and between white and black rhinoceros' species were evaluated using a students T-test. The left and right eye evaluation was used to identify any operator discrepancy and no substantial difference was foreseen. For pathological changes both per eye and per animal prevalence estimates were explored. Statistical analysis was performed using R (R Core Team, Vienna, Austria) ({{R Core Team}}, 2021).

The data is available on the following repository:
https://github.com/connectAnimalHealth/burger_ocular_rhino_data

4.7 Ethical considerations

An application to the Research Ethics Committee and Animal Ethics Committee of the University of Pretoria was submitted and approval was obtained on 02 July 2021, (Appendix 1 and 2). Written consent was obtained from all owners or their legal representatives. The study coincided with immobilisation of animals for veterinary procedures, translocation and/or health examinations and no animals were immobilised for the sole purpose of this study. Data capture was opportunistic and only

non-invasive ophthalmic examination techniques were utilised. This reduced the ethical and welfare burden of performing this study independently. Data collection was aborted when the wildlife veterinarian, at any stage, felt the physiological parameters of the animal necessitated immediate recovery from immobilisation.

Chapter 5
RESULTS

5.1 General results

Seventy-three animals were examined during the data collection period extending over a period of 1 year, 8 months and 7 days, between 05 October 2021 and 12 June 2023. Data collection was dependent on availability of suitable animals undergoing veterinary procedures as described previously. Animals were examined at five separate locations in the Gauteng, Limpopo, and North-West provinces in the northern parts of South Africa. Five animals were examined in the greater Dinokeng area (6.8%), 23 animals in the Pilanesberg National Park (31,5%), 12 animals in the Rietvlei Nature Reserve (16.4%), 14 animals in the greater Rust de Winter area (16.4%), and 19 animals in the greater Thabazimbi area (26%). Of the examined animals, 68 were white rhino (93.2%) and 5 were black rhino (6.8%). Twenty-four animals were male (32.9%), and 49 animals were female (67.1%). The age distribution of the population examined includes 3 calves (4.1%) 3 sub-adults (4.1%) and 67 adult animals (91.8%). Thirty-three animals (45.2%) were captive in camps on game farms and 40 animals (54.8%) were free roaming in nature reserves or national parks. Five animals (6.8%) underwent immobilization for horn-transmitter implantation and 68 animals (93.2%) underwent immobilization for horn reduction. Sixty-eight animals underwent chemical immobilization with a combination of etorphine and azaperone and 5 animals underwent immobilization with etorphine, azaperone and medetomidine. Intravenous butorphanol was given to all animals and oxygen insufflation provided when possible.

The following pathologies were excluded from analysis of the normative data for STT, IOP, and biometry: follicular conjunctivitis, corneal foreign body, keratitis, corneal ulcer, and cataract.

An evaluation of the overall prevalence of animals with any pathology showed a prevalence of 0.56 (n = 41) (N = 73) with a 95% confidence interval of between 0.44 to 0.68.

An evaluation of the overall prevalence of left eyes with any pathology showed a prevalence of 0.4(n = 29) (N = 73) with a 95% confidence interval of between 0.28 to 0.52. An evaluation of the overall prevalence of right eyes with any pathology showed a prevalence of 0.44(n = 32) (N = 73) with a 95% confidence interval of between 0.32 to 0.56. An evaluation of the overall prevalence of individual eyes with any pathology showed a prevalence of 0.42(n = 61) (N = 146) with a 95% confidence interval of between 0.34 to 0.5.

5.2 Descriptive statistics for Schirmer Tear Test (STT)

The mean estimate for the right eye STT is 19.09 mm/min (95% CI:17.48 – 20.69 mm/min). An evaluation of the comparison between black and white rhino showed a mean STT in the black rhino group of 17.5 mm/min. The mean STT in the white rhino group is 19.16 mm/min with no difference between species (P = 0.63).

The mean estimate for the left eye STT is 17.64 mm (CI: 16.14 – 19.14 mm/min). An evaluation of the comparison between black- and white rhino showed a mean STT in the black rhino group of 13.67 mm/min. The mean STT in the white rhino group is 17.91 mm/min, with no difference between species (P = 0.33).

No significant difference between left and right eye was established (P = 0.16)

	Mean	SD	Minimum	Median	Maximum
STT OD	19.09	5.47	8.00	19.00	34.00
STT OS	17.64	5.11	7.00	17.00	30.00

Table 5. Specific estimate of the Schirmer Tear Test (STT) with 95% Confidence interval. Values are in mm/min. OD Right eye; OS Left eye; SD Standard deviation.

5.3 Descriptive statistics for Intraocular Pressure (IOP)

The mean estimate for the right eye IOP is 41.12 mmHg (CI: 36.74 – 45.51 mm/Hg). An evaluation of the comparison between black- and white rhino showed a mean in the black rhino group of 25.75mm/Hg. The mean in the white rhino group is 41.93 mmHg with no difference between species (P = 0.05).

The mean estimate for the left eye IOP is 42.66 mmHg (95% CI: 38.52 – 46.81 mmHg). An evaluation of the comparison between black- and white rhino showed a mean in the black rhino group of 30.33 mm/Hg. The mean in the white rhino group is 43.59 mmHg, with no difference between species (P = 0.15).

No significant difference between left and right eye was established (P = 0.92).

	Mean	SD	Minimum	Median	Maximum
IOP OD	41.12	13.71	22.50	39.50	69.00
IOP OS	42.66	13.74	21.00	42.50	75.50

Table 6. Specific estimate of intraocular pressure (IOP) with 95% Confidence interval. Values are in mm/Hg. OD Right eye; OS Left eye; SD Standard deviation.

5.4 Descriptive statistics for Keratometry

5.4.1 Corneal curvature in mm

The mean estimate for the right eye corneal curvature is 11.07 mm (95% CI: 10.85 – 11.3 mm).

The mean estimate for the left eye corneal curvature is 10.9 mm (95% CI: 10.56 – 11.24 mm).

Collection of a full data set (left and right eye) was not possible for the black rhino group thus an evaluation of the comparison between black- and white rhino is not possible.

No significant difference between left and right eye was established (P = 0.55).

	Mean	SD	Minimum	Median	Maximum
R1R2 AVG OD	11.07	0.50	10.06	11.16	11.81
R1R2 AVG OS	10.90	0.80	7.74	11.24	11.58

Table 7. Specific estimate of corneal curvature in mm with 95% confidence interval. Values are in mm. OD Right eye; OS Left eye; SD Standard deviation.

5.4.2 Corneal curvature in dioptre (D)

The mean estimate for the right eye corneal curvature is 30.55 D (95% CI: 29.91 – 31.18 D).

The mean estimate for the left eye corneal curvature is 31.16 D (95% CI: 29.92 – 32.41 D).

Collection of a full data set (left and right eye) was not possible for the black rhino group thus an evaluation of the comparison between black- and white rhino is not possible.

No significant difference between left and right eye was established (P = 0.52).

	Mean	SD	Minimum	Median	Maximum
K1 K2 AVG OD	30.55	1.39	28.72	30.25	33.50
K1 K2 AVG OS	31.16	2.95	29.12	30.00	43.62

Table 8. Specific estimate of corneal curvature in dioptre with 95% confidence interval. Values are in D. OD Right eye; OS Left eye; SD Standard deviation.

5.4.3 Intraocular lens power

The mean estimate for the IOL power in the right eye, using the Binkhorst method, is 25.26 D (95% CI: 23.86 – 26.67 D). The mean estimate for the IOL power in the left eye, using the Binkhorst method, is 24.09D D (95%CI: 22.21 – 25.97 D).

The mean estimate for the IOL power in the right eye, using the Retzlaf method, is 24.9 D (95%CI: 23.49 – 26.3 D). The mean estimate for the IOL power in the left eye, using the Retzlaf method, is 23.73 D (95%CI: 21.84 – 25.62 D).

Collection of a full data set (left and right eye) was not possible for the black rhino group thus an evaluation of the comparison between black- and white rhino is not possible.

No significant difference between left and right eye was established for the Binkhorst method (P = 0.83).

No significant difference between left and right eye was established for the Retzlaf method (P = 0.81).

	Mean	SD	Minimum	Median	Maximum
IOL power OD: Binkhorst	25.26	3.09	21.58	24.46	34.36
IOL power OS: Binkhorst	24.09	4.45	11.99	23.78	31.71
IOL power OD: Retzlaf	24.90	3.09	21.22	24.13	33.97
IOL power OS: Retzlaf	23.73	4.47	11.47	23.43	31.32

Table 9. Specific estimate of IOL (intraocular lens) power for Binkhorst and Retzlaf methods with 95% confidence interval. Values are in dioptre. OD Right eye; OS Left eye; SD Standard deviation.

5.5 Descriptive statistics for Slitlamp Biomicroscopy

Keratitis was the most common ocular pathology observed in 23 rhinos (31.51% (95% CI: 21.13% - 43.44%) and 33 eyes (22.6%) in total. Ten rhinos (20 eyes) had keratitis in both eyes (13.7%), 6 rhinos had keratitis in the right eye (8.22%), and 7 rhinos had keratitis in the left eye (9.59%).

Corneal scarring was the second most common ocular pathology observed in 12 animals (16.44%; 95% CI: 8.79% - 26.95%) and 15 eyes (10.27%) in total. Three animals (6 eyes) had corneal scarring in both eyes (4.11%), 5 animals had corneal scarring in the right eye (6.85%) and 4 animals had corneal scarring in the left eye (5.48%).

Cataract was present in 11 animals (15.07%; 95% CI: 7.77% - 25.36%) and 14 eyes (9.59%) in total. Cataract was observed in both eyes of 3 animals (6 eyes, 4.11%), 3 animals had cataract in the right eye (4.11%) and 5 animals had cataract in the left eye (6.85%).

Corneal ulceration was present in 7 animals (9.59%; 95% CI: 3.94% - 18.76%) 9 eyes (6.16%) in total. Two animals had corneal ulcers in both eyes (4 eyes, 2.74%), 4 animals had corneal ulcers present in the right eye (5.48%) and 1 animal had a corneal ulcer in the left eye (1.37%). All the ulcers appeared to be new and related to the immobilisation attempt.

Pigmentary keratitis was present in 3 animals (4.11%; 95% CI 0.86% - 11.54%) and 5 eyes (3.42%) in total. Two animals had pigmentary keratitis in both eyes (4 eyes, 2.74%) and 1 animal had pigmentary keratitis in the right eye (1.37%).

Two animals had corneal foreign bodies in the right eye (2.74%; 95% CI: 0.33% - 9.55%).

Posterior synechia was present in 2 animals (2.74%; 95% CI: 0.33% - 9.55%) and 3 eyes (2.05%) in total. One animal had posterior synechiae in both eyes (2 eyes, 1.37%) and one animal had posterior synechiae in the right eye (1.37%).

Persistent pupillary membranes were present in two animals (2.74%; 95% CI: 0.33% - 9.55%) 3 eyes (2.05%) in total. Both eyes of one animal (1.37%) and in the right eye of one animal (1.37%) were affected.

One animal had anterior synechiae in the left eye (1.37%; 95% CI: 0.03% - 7.4%)

One animal had follicular conjunctivitis present in both eyes (1.37%; 96% CI 0.03% - 7.4%).

One animal had nuclear sclerosis present in both eyes (1.37%; 96% CI 0.03% - 7.4%).

5.6 Descriptive statistics for Fluorescein stain

Seven animals, and 9 eyes, had positive fluorescein uptake. Six eyes had positive fluorescein stain uptake in the right eye (8.2%), and 3 eyes had positive fluorescein uptake in the left eye (4.1%).

5.7 Descriptive statistics for Ocular ultrasound and biometry

5.7.1. Axial globe length (AGL)

The mean estimate for the right eye axial globe length is 26.2 mm (95% CI: 25.7 – 26.6 mm). An evaluation of the comparison between black- and white rhino showed a mean in the black rhino group of 26.8 mm. The mean in the white rhino group is 26.1 mm, a significant difference ($P<0.05$). The mean estimate for the left eye axial globe length is 26.0 mm (95% CI: 25.5 – 26.5 mm). An evaluation of the comparison between black- and white rhino showed a mean in the black rhino group of 25.8 mm. The mean in the white rhino group is 26.0 mm with no difference between species ($P = 0.75$).
No significant difference between left and right eye was established. ($P = 0.51$).

	Mean	SD	Minimum	Median	Maximum
AGL OD	26.2	1.6	20.9	26.3	29.0
AGL OS	26.0	1.6	21.6	26.3	28.6

Table 10. Specific estimate of axial globe length (AGL) with 95% confidence interval. Values are in mm. Values are in D. OD Right eye; OS Left eye; SD Standard deviation.

5.7.2 Anterior chamber depth (ACD)

The mean estimate for the right eye anterior chamber depth is 2.7 mm (95% CI: 2.5 – 2.8 mm). An evaluation of the comparison between black- and white rhino showed a mean in the black rhino group of 3.3 mm. The mean in the white rhino group is 2.6 mm with no difference between species (P = 0.14).
The mean estimate for the left eye anterior chamber depth 2.7 mm (95% CI: 2.5 – 2.8 mm). An evaluation of the comparison between black- and white rhino showed a mean in the black rhino group of 2.7 mm. The mean in the white rhino group is 2.7 mm with no difference between species (P = 0.81).
No significant difference between left and right eye was established. (P = 0.98).

	Mean	SD	Minimum	Median	Maximum
ACD OD	2.7	0.6	1.0	2.8	3.6
ACD OS	2.7	0.6	1.3	2.8	3.6

Table 11. Specific estimate of anterior chamber depth (ACD) with 95% confidence interval. Values are in cm. Values are in D. OD Right eye; OS Left eye; SD Standard deviation.

5.7.3 Crystalline lens thickness (CLT)

The mean estimate for the right eye crystalline lens thickness is 6.5 mm (95% CI: 6.4 – 6.6 mm). An evaluation of the comparison between black- and white rhino showed a mean in the black rhino group of 6.0mm. The mean in the white rhino group is 6.5 mm, a significant difference (P<0.05).
The mean estimate for the left eye crystalline lens thickness is 6.4 mm (95% CI: 6.3 – 6.6 mm). An evaluation of the comparison between black- and white rhino showed a mean in the black rhino group of 6.2 mm. The mean in the white rhino group is 6.4 mm with no difference between species (P = 0.27).
No significant difference between left and right eye was established. (P = 0.32).

	Mean	SD	Minimum	Median	Maximum
CLT OD	6.5	0.4	5.4	6.6	7.1
CLT OS	6.4	0.5	4.8	6.5	7.2

Table 12. Specific estimate of crystalline lens thickness (CLT) with 95% confidence interval. Values are in mm. Values are in D. OD Right eye; OS Left eye; SD Standard deviation.

5.7.4 Posterior segment depth (PSD)

The mean estimate for the right eye posterior segment depth is 16.3 mm (95% CI: 16.0 – 16.6 mm). An evaluation of the comparison between black- and white rhino showed a mean in the black rhino group of 16.3 mm. The mean in the white rhino group is 16.3 mm with no difference between species (P = 0.94).
The mean estimate for the left eye posterior segment depth 16.2 mm (95% CI: 16.0 – 16.5 mm). An evaluation of the comparison between black- and white rhino showed a mean in the black rhino group of 15.9 mm. The mean in the white rhino group is 16.3 mm with no difference between species (P = 0.55).

No significant difference between left and right eye was established. (P = 0.63).

	Mean	SD	Minimum	Median	Maximum
PSD OD	16.3	1.0	13.7	16.4	19.1
PSD OS	16.2	1.0	13.4	16.3	1.87

Table 13. Specific estimate of posterior segment depth (PSD) with 95% confidence interval. Values are in mm. Values are in D. OD Right eye; OS Left eye; SD Standard deviation.

Chapter 6

DISCUSSION AND LIMITATIONS

6.1 Schirmer Tear Test

The incidence, or even presence, of tear film abnormalities or KCS is not known for rhinoceroses. The variation between the minimum (8 mm/min OD; 7 mm/min OS) and maximum measured (34 mm/min OD, 30 mm/min OS) is considerable and could be an indication of tear film abnormalities in some animals. There was no statistical significance between the left and right eyes or between species (P >0.05).

The STT data in this study is in line with the values reported by *Ofri et al., (2002)* for white rhino and *Bapodra and Wolfe,* for the greater one horned rhino. These mean values also fall within the normal expected limits for canine STT values.

The STT values were determined for 104 warmblood horses in South Africa. Mean STT value for the right eye was 24.4 mm/min and 25 mm/min for the left eye *(R Allen and A Goodhead, pers comm)*. The STT 1 values were obtained for 12 captive Nubian Ibex (*Capra ibex nubiana*), 10 captive Burchell's zebras (*Equus burchelli*) and 5 Arabian oryx (*Oryx leucoryx*) at a zoo in Israel. The mean STT values for the ibex was 13.2 ± 5.1 mm/min, 12.7 ± 4.8 mm/min for the oryx and 23.4 ± 3.4 mm/min for the zebra (Ofri et al., 1999). The difference between the zebra values and those of the ibex and oryx was significant in this study. The STT of the zebra resembles the values obtained in the horse, the authors of this study advised caution against extrapolating STT values from one species to other closely related species (Ofri et al., 1999).

Piccione et al., (2008) evaluated the effect on tear production of gender, time, daily rhythm and comparison of the left and right eye in 18 healthy horses. Schirmer tear tests were performed every 4 hours over a 24-hour period. A statistically significant difference was found between gender and the left and right eye (Piccione et al., 2008).

The animals were blindfolded as soon as possible during the immobilisation event, as described under materials and methods section. It is certainly possible that the blindfold would have an influence on the tear production of the eyes. In most instances, while the STT was measured in the one eye, the blindfold was in contact with the contralateral eye. There was no standard procedure employed in this study regarding which eye was examined first, it depended on which side was more accessible for the particular immobilisation event. Factors that influenced this included terrain, accessibility around other people performing data collection simultaneously and the specific reason for immobilisation, as described elsewhere. This made a standardised approach difficult, and the ocular examination was conducted as possible.

STT measurements were obtained with the animals under chemical immobilisation thus, tear flow measurements should be regarded as estimates (Ofri et al., 2002). The effect of the immobilisation drugs, stress associated with the immobilisation event and environment could all certainly affect the STT values.

6.2 Intraocular pressure

The difference in the intraocular pressure measurements of the black rhino group right eye (mean IOP 25.75 mmHg) and the white rhino group right eye (mean IOP 41.93 mmHg) was insignificant (P = 0.05). The difference in the intraocular pressure measurements of the black rhino group left eye (mean IOP 30.33 mmHg) and the white rhino group left eye (mean IOP 43.59 mmHg) was insignificant (P = 0.15). There was no statistical significance between the left and right eyes (P > 0.05).

Variation in normal IOP pressure has been documented in multiple studies for closely related species within the same family. In the Camelidae family, the measured IOP for the llama *(Llama glama)* was 13.10 ± 0.35 mmHg and 14.85 ± 0.45 mmHg for the alpaca *(Lama pacos)*. The IOP measurements obtained for the scimitar-horned oryx *(Oryx dammah)* was 15.58 ± 1.5 mmHg and 11.76 ± 3.43 mmHg for the closely related Arabian oryx (Oryx leucoryx), both of which are part of the Hippotraginae subfamily (Ofri et al., 2002; Willis et al., 2000).

Indentation and applanation tonometry were performed in 10 Grant zebras *(Equus burchelli)* by *Ofri et al*. Immobilisation of the animals was achieved using a combination of etorphine hydrochloride and acepromazine maleate. The mean IOP for indentation tonometry of 20 eyes were 25.30 ± 3.06 mmHg and the mean IOP in six eyes with applanation tonometry was 29.47 ± 3.43 mmHg (Ofri et al., 1998b). The immobilisation protocol in the *Ofri et., al 1998* study resembles the protocol used during this study.

The mean IOP, using the TonoVet and TonoVet Plus, was estimated in 30 horses (26 warmbloods, 4 coldbloods; 19 geldings and 11 mares) and 60 eyes. The IOP's were estimated without using any sedation, local anaesthetic blocks, or topical ocular anaesthesia. The mean obtained IOP readings were 21.0 ± 3.14 mmHg (range: 14.40 – 27.20 mmHg) for the TonoVet (Mustikka et al., 2020).

The mean IOP values obtained for 104 warmblood horses in South Africa was 36.5 mmHg for the right eye and 32.6 mmHg for the left eye. No sedation or local anaesthesia were used in any of these animals during their examination *(R Allen and A Goodhead, pers comm).*

Intraocular pressure estimates were measured in 57 miniature donkeys (Equus africanus asinus) using the TonoVet. Thirty-five of the animals received intramuscular xylazine hydrochloride 10% together with auriculopalpebral nerve blocks before measuring the IOP's and 22 animals received no sedation or nerve block. The mean IOP for the 114 eyes were 25.75 ± 5.70 mmHg (range: 14.34 – 37.15 mmHg) with rebound tonometry (Hibbs et al., 2019).

The mean IOP estimates of these 5 studies are summarised below:

	Mean IOP	SD	Minimum	Maximum
Horses	21.0	3.14	14.40	27.20
Miniature donkey	25.75	5.70	14.34	37.15
Grant Zebra: Indentation	25.30	3.06	-	-
Grant Zebra: Applanation	29.47	3.43	-	-
White Rhino	32.1	10.4	-	-
Greater one-horned rhino	32.2	6.62	-	-

Table 14. Comparison of IOP in multiple species. Values are in mmHg. IOP Intraocular pressure; mmHg millimetres mercury.

The mean IOP measurements in this study is significantly higher than the values reported by *Bapodra and Wolfe, (2014)* and *Ofri et al., (2002)*, the animals in these two studies were zoo kept and thus, most probably, easier to immobilize with less physiological stress placed on the animals during the immobilisation event. Immobilising rhino in field conditions is never simple or straightforward. A considerable amount of manoeuvring, from ground or air, is required to get the wildlife veterinarian in a suitable position to fire the dart and the animals often have to be coaxed away from unsuitable- or dangerous terrain (rocky outcrops, deep ravines, water dams, predators, other large game), before the immobilisation attempt, to prevent injury or self-injury. Further, most of the animals have been immobilised on previous occasions and it is not unusual for the animals to react to the noise of a helicopter. This results, in some instances, in animals actively avoiding the immobilisation attempt by running into thick bush/wooded areas or simply constantly running away from the vehicle or helicopter. It would be reasonable to assume that these factors would have an impact on the physiologic state of the animal with increased fight and flight response and associated increases in heart rate, blood pressure and breathing rate expected. These factors may lead to changes in IOP as discussed previously.

The induction of rapid immobilisation by etorphine (mu-agonist opioid) is characterised by respiratory depression, hypoventilation, hypoxaemia, hypercapnia and acidaemia (Bush et al., 2004; de Lange et al., 2017; Kock and Burroughs, 2012; Meuffels, 2022; Portas, 2004; Sandra Wenger et al., 2007). Thoracic muscle rigidity is another feature of the immobilisation and this has a profound effect on the respiration (Portas, 2004). While immobilized, the rhinoceros globes are very prominent, in what is best described as “bulging”. The eyelids are open involuntarily and ocular protection against the environment is always required. Respiratory effort is considerably increased, and the animals take large, heavy breaths between periods of apnoea/hypoventilation. The muscle rigidity is considerable and the effect of the immobilisation drugs on the extra-ocular muscles (EOM) is not known, contraction of the EOM could have a significant impact on IOP. These factors could, in part, explain the elevated IOP measurements during rhino immobilisation.

The effect of sedative drugs on the IOP of normal, healthy horses has been evaluated. The sedative protocols evaluated in these studies includes the following: xylazine & butorphanol; detomidine & butorphanol; detomidine alone; xylazine alone and romifidine alone. The IOP was lowered in all the research subjects in these studies (Holve, 2012; Joyner et al., 2021; Stine et al., 2014; Van Der Woerdt et al., 1995).

Head position and its effect on IOP was evaluated in 30 horses. The animals were sedated with detomidine, and the intraocular pressures were measured with the head in two positions – above the heart and below the heart. Head position had a significant effect on the measured IOP with IOP raised in the head down position (Alling et al., 2021).

In glaucomatous eyes, retinal ganglion cell death is associated with reduced optic nerve axoplasmic flow and compromised optic nerve circulation (Brooks et al., 1999a). It has been shown in normal dogs that 10% of optic nerve axons have mild obstruction to axoplasmic flow at an IOP of 25 mmHg. The obstruction to axoplasmic flow was virtually 100%, in normal dogs, with an IOP of 50 mmHg (Brooks et al., 1999a). The retinal ganglion cell axons are provided with nutrition and mechanical support by the

lamina cribrosa as they form the optic nerve in the scleral canal (Brooks et al., 1999b). The lamina cribrosa in the adult horse is similar to the dog and exists as a complex, multilayered structure composed of astrocytes, an extracellular matrix, myelinated optic nerve axons and capillaries (Brooks et al., 1999b, 2000). The resilience of the equine lamina cribrosa is suggested to be a function of its macromolecular structure and it may be that this is why the equine eye displays the ability to maintain vision despite chronic elevations of IOP (Allbaugh et al., 2016; Brooks et al., 2000). It is certainly worth considering that the rhino eye may have a similar lamina cribrosa structure to the horse and thus a similar ability to tolerate high IOP's.

No data on the corneal and scleral thickness and rigidity is available for rhinos, and these factors may play an additional role in the elevated IOP measurements obtained in these animals. It is well described in human ophthalmology that for every 10µm increase corneal thickness, as measured with ultrasonic pachymeter, there is a corresponding increase in measured IOP, which differs somewhat depending on the tonometer used (Bhan et al., 2002; Browning, 2004).

The validity and accuracy of rebound tonometry in rhino is obviously not known. It would be ideal if applanation and rebound tonometry could be combined during any future studies on rhino, and, if possible, to validate both against manometry should an enucleated rhino eye ever be available for this purpose. It is also not known if the rebound tonometer overestimated the IOP measurements in this study, this could be an additional factor that may have contributed to the higher IOP estimates in this study.

Rebound tonometry was not performed on eight animals due to tonometer dysfunction on the particular day, a spare tonometer was not available. Three rebound tonometer readings were obtained for 50 rhinos, and two readings were obtained for 23 animals. In instances where 3 readings were obtained, the middle, or second, reading was used as part of the data set. In instances where 2 readings were obtained, the two measurements were averaged, and this was used in the data set.

6.3 Keratometry

Meister et al., (2018) determined the refractive state, average corneal curvature, and biometry, for calculation of the IOL power, for the following horse breeds:

	Corneal curvature D	C (ACD/0.73) mm	AGL mm
Halflinger	21.30 ± 0.56	9.30 ± 0.54	39.43 ± 1.26
Friesian	20.02 ± 0.60	10.12 ± 0.33	42.23 ± 1.00
Pony	22.61 ± 1.76	8.68 ± 0.78	38.85 ± 3.13
Shetland Pony	23.77 ± 0.94	8.71 ± 0.81	37.21 ± 1.50
Warmblood	20.76 ± 0.88	9.39 ± 0.51	40.65 ± 1.30

Table 15. Corneal curvature and biometry for different horse breeds.

Determining the mean IOL power across all horses, using the Binkhorst formula, is 18.73 D and 18.54 D utilizing the Retzlaff formula.

Gilger et al., (2006) determined the mean PACD (post-operative anterior chamber depth = cornea to 0.5 x CLT), AGL and IOL power for fourteen horses as described previously and summarised below:

	Corneal curvature (mean) D	Pre-op ACD mm	PACD mm	AGL mm	Predicted IOL power: Binkhorst D	Predicted IOL power: Retzlaff D
Horse	16.46 ± 1.50	5.63 ± 0.86	11.50 ± 0.95	39.23 ± 1.26	29.92 ± 2.50	29 ± 2.52
Miniature foal	16.46 ± 1.50	3.35	6.76	26.93	31.84	31.35
Belgian	16.46 ± 1.50	6.72	12.64	43.10	31.36	28.69

Table 16. Corneal curvature, biometry and IOL power for different horse breeds. IOL intraocular lens.

The biometry of the rhinoceros eye differs to the measurements in these horse studies with a shorter axial globe length of 26.3 ± 1.5mm (OD) and 26.3 ± 1.7 mm (OS). The miniature foal is the only species described here with an AGL that approximates that of the rhino (26.93 mm). The anterior chamber depth of 2.7 ± 0.5mm (OU) is also much shallower than the values obtained in the horse studies with a PACD of 5.95 mm OU in the rhinos, the closest approximation again being the miniature foal.

The mean calculated IOL power values for rhinos in this study falls between the values obtained for the horses in the respective studies. The mean corneal curvature of rhinos (11.07 ± 0.50 mm OD; 10.90 ± 0.80 mm OS) is also considerably flatter than the corneal curvature measured for dogs in the study by *Kawasaki et al., (2020)* where the mean corneal curvature ranged from 7.54 ± 0.30 mm in Pomeranians to 9.28 ± 0.19 mm in golden retrievers. The AGL was determined for 20 mesocephalic mix breed dogs at 52 weeks of age using B-mode ultrasonography with a mean result of 19.52 ± 0.18 mm (Tuntivanich et al., 2007). The longer axial globe length together with the flatter corneas of rhinos will result in an eye that requires less refraction of light to obtain a clear retinal image, and this will translate into less required lenticular refraction. For this reason IOL power value for rhinos are lower (less refraction) compared to dogs, where the standard of care to achieve emmetropia is placement of a 41 D IOL (Gilger, 1997; Glover and Constaninescu, 1997; Ozgencil, 2005; Sigle and Nasisse, 2006; Williams et al., 1996). In this context, the IOL power for horses in the *Meister et al., (2018)* is less than what is predicted for rhinos, also due to their flatter corneal curvature and longer AGL (compared to rhinos) resulting in less required refraction to adequately focus light on the retina.

In human practice, auto ref-keratometers, whether hand-held or standalone, is not as accurate an instrument compared with subjective manual refraction (skiascopy) or manual keratometry and is used as an objective guideline to provide information. The human cornea is at its most spherical, in terms of curvature, in the very centre of the cornea and visual axis and this is where the corneal curvature is measured. Human patients would fixate on a specific target to achieve this *(M Horowitz, BVSc (UP), BOptom (RAU), MCOptom, pers comm)*. This degree of precision was not possible in this study and possible measurement errors could be present in the data. Corneal topography would provide more accurate data for corneal curvature measurements, but this is not practical in field conditions. Rhino corneas are flatter (less curvature) than what is seen in humans where curvatures are roughly between 7.5 – 8.5 mm *(M Horowitz, BVSc (UP), BOptom (RAU), MCOptom, pers comm)*. Performing manual refraction is not possible in field conditions.

Complete keratometry data (left and right eye) were obtained for 14 animals in total. In some animals a reading could be obtained in one eye only and in others no readings were obtained at all. The keratometer was a loan unit and thus was not always available for use either. More accurate data would

be needed to ensure the IOL power values obtained here is within range to provide emmetropia in the event of cataract extraction surgery.

6.4 Observed ocular pathology

The examination with the slitlamp biomicroscope started with an overview of the eyelids, third eyelid, conjunctiva, cornea, iris and anterior lens. The eyelids are heavy and folded with thick skin, as for the rest of the rhino. Multiple, long eyelash like hair is present on the superior eyelid and shorter hair is present on the inferior eyelid. The conjunctiva is dark pink to red in areas and appears more thickened than what is seen in domestic animals, the third eyelid is thick and fleshy with variable amounts of pigment. The iris is a dark brown with a round pupil opening with no *granula iridica*. The fundus is atapetal and a dull brown and resembles the paurangiotic fundus of the horse with an oval shaped optic nerve head.

Figure 8. Rhino eye illustrating the eyelids and corneal surface.

Conjunctiva

One animal had bilateral conjunctival lesions that appeared similar to typical follicular conjunctivitis seen in domestic animals. The lesions were focal, round, raised, smooth and pink in colour and affected the temporal bulbar conjunctiva in both eyes.

Cornea

One subadult female had a small focal corneal scar in the right eye. One adult female had a linear, mid stromal, corneal scar in the right eye. One subadult male had a posterior stromal corneal scar in the right eye. One adult female had a small fibrotic posterior stromal corneal scar in the right eye. One adult female had a linear vertical, fibrotic scar of the ventral axial cornea in the right eye. Two adult females and two adult males had limbal vascular keratitis in both eyes. One adult male and 3 adult females had

only limbal keratitis in the right eye. Two adult females and 1 adult male had limbal keratitis in the left eye. One adult male had limbal vascular keratitis and limbal pigmentary keratitis in both eyes. One adult female had limbal vascular keratitis and a lateral axial, linear, horizontal, fibrotic scar of the left eye. One adult female had limbal pigmentary keratitis in both eyes, limbal vascular keratitis in the right eye, a linear corneal scar in the right eye and an axial corneal scar in the left eye. One adult black rhino female had limbal vascular keratitis of both eyes and a fibrotic, superficial corneal scar in the left eye. One adult female had a superficial corneal ulcer in the right eye. One adult female had a small superficial epithelial ulcer in the right eye. One adult male had limbal vascular keratitis in both eyes and superficial ventral corneal epithelial ulcers in both eyes. One adult female had limbal vascular keratitis in both eyes and superficial corneal epithelial trauma evident as multiple, linear superficial corneal epithelial lacerations, these were most probably associated with the immobilisation event while the animal ran through thick bush. One adult male had thorn/plant material foreign body in the dorsal limbal cornea with associated vascular keratitis in the right eye, the inflammatory reaction to this foreign body was remarkably mild and the eye was normal in all other respects, the left eye had limbal vascular keratitis. One adult male had mild, central axial, superficial epithelial trauma in both eyes and a thorn/plant foreign body in the dorso-lateral paraxial cornea, the inflammatory reaction around this foreign body was also minimal.

Iris

One adult male had small pupil margin persistent pupillary membranes in both eyes. One adult female had small, anterior synechiae in the tempero-ventral cornea at the 4-clock position of the left.

Lens

One adult male had a posterior capsular opacity in the lens of the left eye. One adult male (“Sierra”) had cataracts in both eyes, the right lens had a hyperechoic cortex and nucleus on ultrasound and the left lens was anterior-posteriorly flattened with a hyperechoic cortex and nucleus.

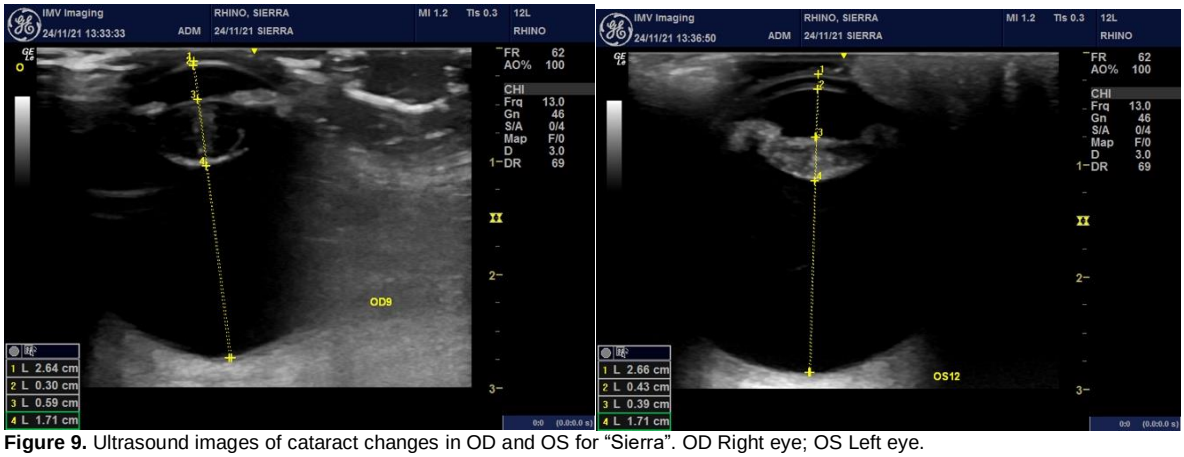

Figure 9. Ultrasound images of cataract changes in OD and OS for “Sierra”. OD Right eye; OS Left eye.

One adult male black rhino (“Nr 71”) had nuclear sclerosis of both lenses.

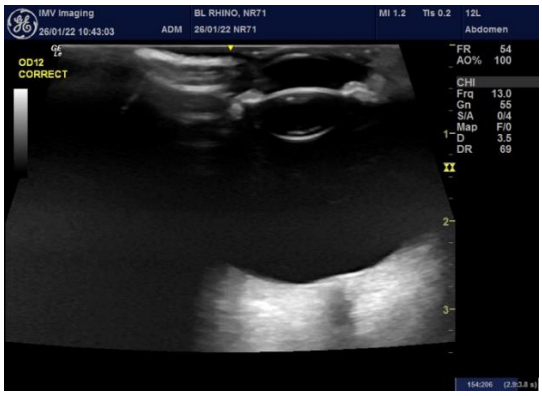

Figure 10. Nuclear sclerosis of lens in “Nr 71”.

One adult male had a focal, anterior subcapsular cataract opacity at the 5-clock position of the lens. One adult female (“Nr 17”) had a cortical cataract and vitreal degeneration of the left eye, on ultrasound the lens capsule margins were irregular, as was the lens shape and the entire lens was hyperechoic.

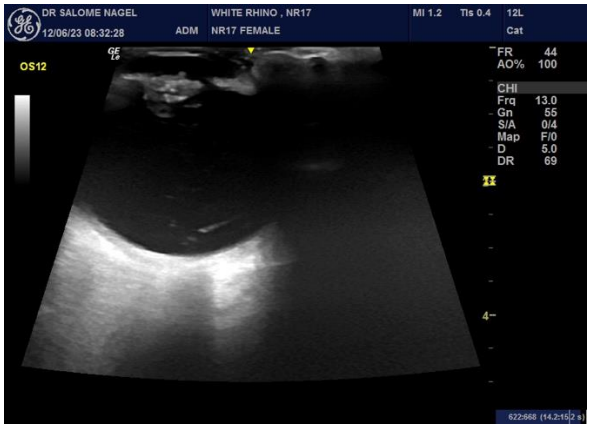

Figure 11. Cataract changes of OS in “Nr 17”. OS Left eye.

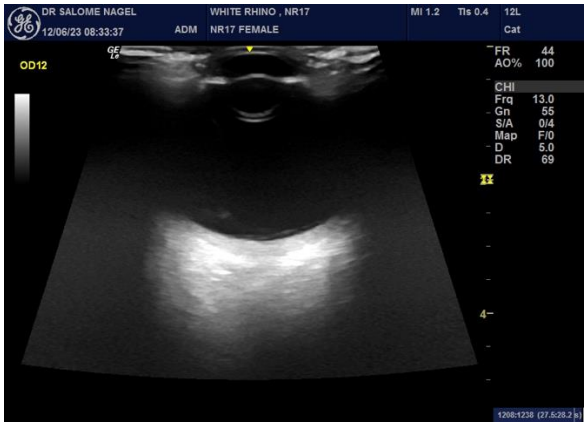

Figure 12. Normal OD of “Nr 17”. OD Right eye.

One adult female had cataracts in both lenses that presented as posterior capsular, ring-like opacities that resembled posterior capsular opacity.

Cornea and conjunctiva

One adult female had a granuloma like nodule on the superior conjunctiva with limbal vascular keratitis in the right eye and an iatrogenic superficial corneal ulcer in the left eye.

Cornea and iris

One adult female had limbal vascular keratitis in both eyes, and a traumatic corneal ulcer and persistent pupillary membrane in the right eye. One adult male had a lateral paraxial fibrotic corneal scar and posterior synechiae at the 4-5 clock position of the iris in the right eye and linear, diagonal, fibrotic corneal scar in the left eye.

Cornea and lens

One adult male ("Hotel") had bilateral, "steel wool"-like cataracts in both eyes, limbal vascular keratitis in both eyes and limbal pigmentary keratitis in the left eye, the ultrasound image of the lenses showed hyperechoic, subcapsular, concentric rings.

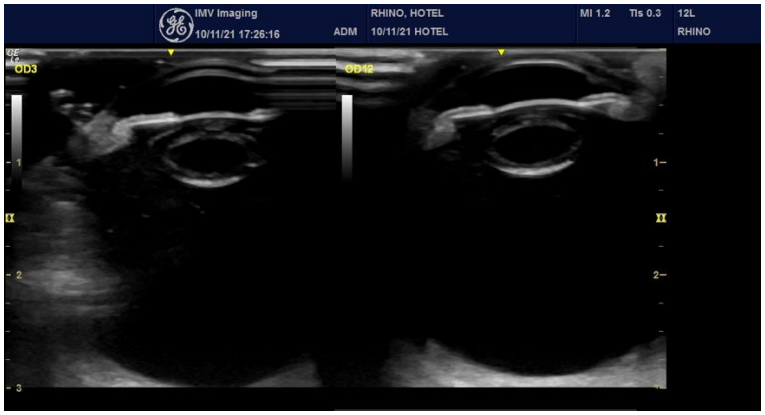

Figure 13. Cataract changes in "Hotel".

One adult female had a small, axial, anterior subcapsular cataract in the right eye and limbal vascular keratitis in the left eye. One adult male had a medial paraxial, posterior stromal fibrotic corneal scar associated with a linear, anterior subcapsular cataract and some pigment deposition was evident on the anterior lens capsule. One black rhino male had small, multifocal, anterior subcapsular cataract opacities in both eyes and mild, central axial- and limbal keratitis in the left eye. One adult female had an incipient, anterior cortical cataract of the medial paraxial lens in the left eye and a small, medial paraxial, linear, fibrotic corneal scar associated with the cataract, also of the left eye.

Cornea, iris and lens

One adult female had a small, linear, medial paraxial fibrotic corneal scar and posterior synechiae in the right eye and a fibrotic corneal scar with associated posterior synechiae and a focal cataract in the ventro-lateral quadrant of the left eye. One adult female ("Nr 35") had severe keratitis with a well-developed vascular component and a mature cataract in the left eye, the iris leaflets were thickened. On ultrasonography, the lens was practically absent, and the globe was filled with a large, irregular, hyperechoic structure which virtually obliterated the anterior chamber.

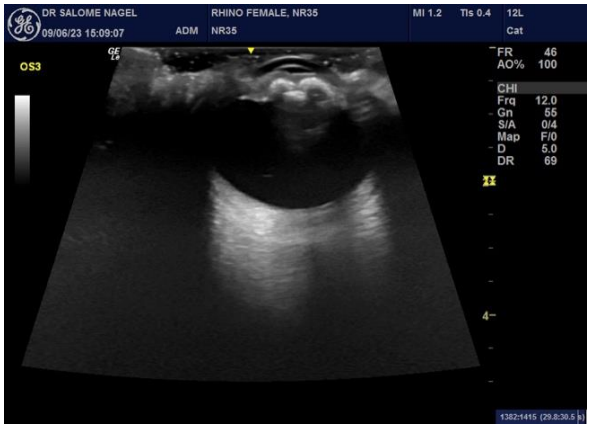

Figure 14. Ultrasound image adult female “Nr 35” OS pathology. OS Left eye.

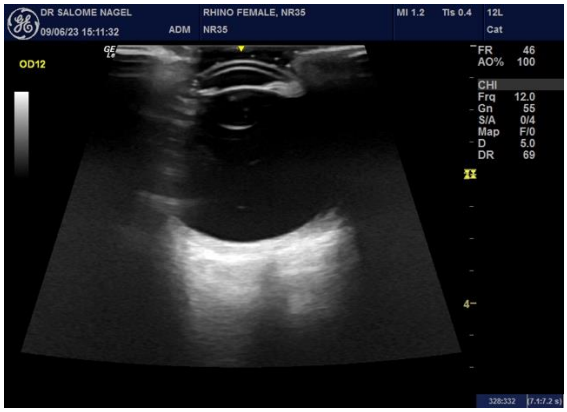

Figure 15. Ultrasound image adult female “Nr 35” OD normal eye. OD Right eye.

The limbal keratitis observed was usually mild and was confined to the limbal cornea with small, straight, individual blood vessels extending 1-2 mm into the surrounding cornea from the limbus. There was no apparent cause for the keratitis. The pigmentary keratitis was also confined to the limbal zone of the conjunctiva and cornea. Most of the corneal scarring is probably related to trauma given their natural habitat and propensity for running through obstacles (like bushes) as opposed to around the obstacle. Corneal scarring associated with iris and lenticular pathology occurred in 4 animals (5.48%). None of the animals suffering from cataract showed any obvious signs of decreased vision considering that they were able to negotiate their environment with apparent ease. The animals with the corneal foreign bodies had remarkably little inflammatory reaction around the organic material, the presence of organic material on, or in, the cornea of domestic pets elicits, in my clinical experience, a severe keratitis that would lead to loss of the affected globe if the situation were not rectified.

The corneal ulcers appeared to be new and associated with the capture event itself, either from the blindfold or during the actual immobilisation attempt. The blindfold is the only factor that the veterinarian has any control over, care should be taken to ensure the globes are always protected. The corneal ulcer data was removed from the overall prevalence of pathology reporting considering the iatrogenic nature of the pathology in this study.

The overall prevalence for ocular pathology in rhinoceros’ (56% overall, 40% left eyes, 44% right eyes) in this study is higher than what is found in other free-living wildlife species.

Ocular disease in free-living and captive pinnipeds is common, with corneal lacerations, corneal oedema, or other opacities, cataractous change and lens luxations commonly found in captive pinnipeds. Ocular pathology commonly encountered in wild pinnipeds include ocular trauma and cataract changes. Corneal scars, cataract changes in the area of the lens sutures, cataracts and iris depigmentation were described in wild northern fur seals (*Callorhinus ursinus*) (Miller et al., 2013).

The prevalence of reported ocular disease in 27 000 free-living hummingbirds was shown to be 0.1% (Clark and Russell, 2020). Hummingbird ecology requires them to have a high level of visual processing and loss of their vision may lead to increased mortality rate (Moore et al., 2019). *Moore et al., (2019)* reported the prevalence of ocular disease in free-living hummingbirds to be only 2.28% (Moore et al., 2019).

A prevalence rate of 2.05% for ocular abnormalities was reported in 1510 Long-eared Owls (*Asio otus*) spanning a 22-year period, this is close to what was reported for hummingbirds (Holt and Layne, 2008).

Ocular disease prevalence was reported for 202, non-domestic felids comprising 18 different species, 33 (16.3%) cats and 47 eyes were diagnosed with ocular abnormalities. Ocular lesions in this study included corneal disease (37%) (ulcerations, perforations, keratitis, corneal scars), cataracts (23.9%), hyphema (8.7%), lens luxation (6.5%), retinal detachment (6.5%), uveitis (4.3%), conjunctival disease (4.3%), retinal degeneration (2.1%), glaucoma (2.1%) and optic neuritis (2.1%) (Nguyen et al., 2022).

6.5 Ocular biometry and ultrasound

Ultrasound remains an outstanding imaging modality to visualise and evaluate pathology of the anterior and posterior segment of the globe. The ultrasound images of lenticular pathology were remarkably clear and allowed for adequate biometry measurement data.

The biometry data in this study is very similar to the measurement data in the *Bapodra and Wolfe, (2014)* study for all the measured ocular structures. These values are summarised below:

	AGL	ACD	CLT	PSD
Greater one horned	26.1 ± 1.1	2.8 ± 0.6	7 ± 1.1	14.6 ± 1.3
OD this study	26.2	2.7	6.5	16.3
OS this study	26.0	2.7	6.4	16.2

Table 17. Comparison of mean biometry data for different rhino species. OD Right eye; OS Left eye. AGL axial globe length; ACD anterior chamber depth; CLT crystalline lens thickness; PSD posterior segment depth.

There was a significant difference in AGL between the black and white rhinoceros' right eye ($P < 0.05$) and no significant difference between the black and white rhinoceros left eye ($P > 0.05$). There was also a significant difference in the CLT of the black and white rhinoceros' right eye ($P < 0.05$). The most probable cause for the differences in the right eye is operator technique, especially considering the insignificance of either measurement in the left eyes. The number of black rhinos examined was also low and care should be taken regarding the interpretation of the significance in this instance.

Obtaining ocular biometry data through use of both A- and B-mode ultrasonography should provide more accurate data for future studies

Obtaining accurate measurements in field conditions comes with some degree of challenge, there is a need for haste as multiple procedures are usually performed on the same animal during the immobilisation event.

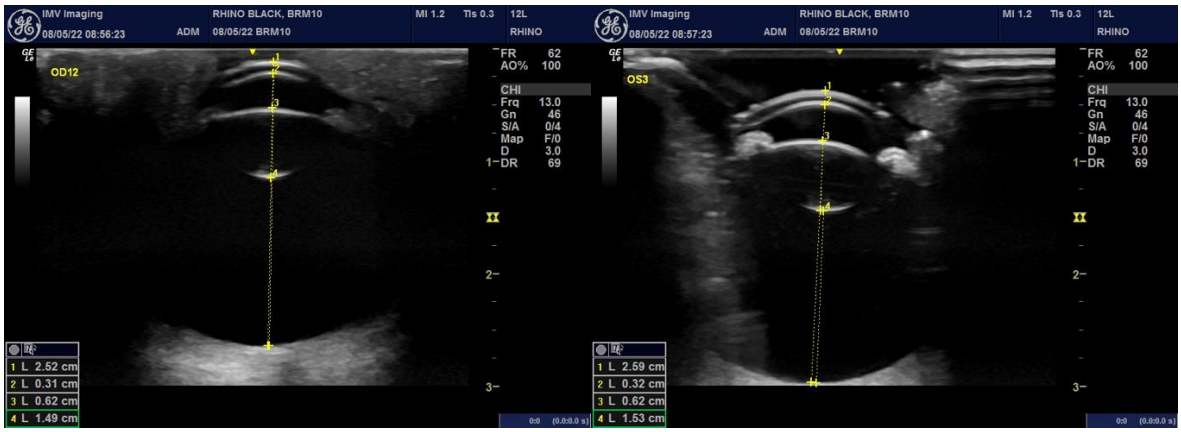

Figure 16. Ultrasound images displaying measurement data for biometry.

6.6 Rhinoceros ecology

The habitat utilised by the African rhinoceroses range from open grasslands to woodlands. The broad, square lips of the white rhinoceros are adapted for grazing in open savannah, and they are strict grazers(Coimbra and Manger, 2017; Radcliffe and Morkel, 2014). *Player and Feely (1960)* listed four basic habitat requirements for the white rhino: 1) areas of short grass, for which they have a marked preference; 2) the availability of water for drinking and in which to wallow; 3) adequate bush cover; and 4) relatively flat terrain (Player, I.C. and Feely, J.M., 1960). The black rhinoceros has a hooked, prehensile lip that facilitates browsing in relatively enclosed woodland habitats (Coimbra and Manger, 2017; Radcliffe and Morkel, 2014). Black rhinos are dependent on a water supply for drinking (they are seldom found more than 10 – 15 km away from it) and wallowing and together with a habitat that provides adequate shrubs and young trees. (Skinner and Chimimba, 2005).

The home ranges of white rhino cows can overlap with the home range of as many as six or seven territorial bulls and overlap with the home ranges of other cows (Owen-Smith, 1975; Pienaar et al., 1993). White rhinos are essentially solitary but loose associations of male and female subadults and adult females, with small calves, can last from days to years (Shrader and Owen-Smith, 2002). The group size can vary between two to five individuals but larger groups of up to fifteen animals have been reported (Owen-Smith, 1975). The territories of neighbouring territorial bulls do not overlap, and these territories are clearly defined and defended from other territorial bulls. The size of the territory varies according to the region the animals inhabit. Territorial, dominant bulls will tolerate the presence of several subordinate bulls within their territory as long as they remain submissive. The subordinates will spend time in these territories but will also make occasional explorations outside it (Owen-Smith, 1975). Black rhinos also tend to be solitary with the bond between cow and calf the only stable relationship. Even this bond is temporary with the adult female chasing off the subadult when she is close to birth of the next calf. Aggregations of subadults into pairs sometimes occurs after separation with their mothers and this can last months to years. Adult females have no strict home range, but they do stay within an area that may overlap with the home range of other black rhinos. The territory of an adult bull is

determined by its dominance status and relative location to water points, female home ranges and other features important to the species (Skinner and Chimimba, 2005).

Although the selection of animals that underwent immobilisation was strictly under the discretion of the owner, park- or reserve official, or attending veterinarian, the characteristic behaviour traits of the animals may in part explain the higher female count (49 animals, 67.1 %) vs males (24 animals, 32.9%). It could also be that females are more prevalent owing to breeding programmes aimed at increasing rhinoceros' numbers.

The white rhino is Africa's second largest land mammal with an adult mass for bulls of 2000-2400 kg and 1600 kg for females. The males are up to 1.8 m tall at the shoulder with females measuring 1.77 m at the shoulder (Skinner and Chimimba, 2005). In contrast, adult black rhinos have a mean weight of around 1000 kg and measure 1.6 m tall at the shoulder. The heaviest weight recorded for a black rhino in the Kruger National Park was 1400 kg and the mean mass for live individuals in the Hluhluwe Game reserve was 852 kg for males and 884 kg for females (Skinner and Chimimba, 2005).

Rhinos are considerably larger than even very large horses and their axial globe length, as a function of their size, seems rather small, compared to the AGL for horses. The axial globe length was measured for 22 capybara's and found to be 22.20 ± 1.71 mm (Montiani-Ferreira et al., 2008b). Ocular biometry was performed in eleven captive bald eagles and the AGL for these birds of prey was 26.57 ± 0.45 mm (Kuhn et al., 2015). Considering these data, it is more probable that the overall globe size is more likely to be a function related to the ecological niche the particular species exploits as opposed to a direct relation to body size.

Chapter 7

CONCLUSIONS

The prevalence of ocular disease, in this subset of animals, is high but appears to have little, if any, adverse effects on their ability to lead a natural life, the best example of this being the blind female who produced offspring. This would be, in my view, in line with survival characteristics of wild animals and speaks volumes of their remarkable ability to adapt to almost any given set of circumstances.
No reports of blepharospasm or lacrimation, as indicators of ocular pain or discomfort, were present for any of the animals observed, even in instances of serious ocular disease which would, in domestic species at least, lead to considerable discomfort, pain and loss of function and/or quality of life. It is also possible that clinical signs of ocular pain were not observed owing to infrequent observation of the animals in their natural habitat or that the signs of ocular pain are subtle and not readily appreciated by distant observers.

Most of the ocular pathology, in this study, seems to be incidental in nature. Keratitis and corneal scarring had the highest prevalence and is most probably related to the environment rhinos inhabit as these are both surface ocular diseases. The corneal ulcerations were all noted to be iatrogenic and associated with either the capture event or with the blindfold applied while immobilised. The presence of the weave pattern of the cloth used as a blind fold on the corneal surface of one examined animal would suggest that current blindfolding techniques could be refined to minimise iatrogenic corneal injury.
The elevated IOP values are difficult to interpret considering the significant physiological stress the animals experience during immobilisation events and the lack of data regarding corneal and scleral thickness and rigidity plus the effect the immobilisation drugs may have on the extraocular muscles.
The absence of significant posterior segment pathology in this study is encouraging.

This first approximation of the IOL power in rhinos would need more accurate data collection procedures should placement of an IOL ever be considered in the species. In horses, a difference in the refractive state of the eye of ± 3 D was calculated for an IOL position of 2 mm anterior or 2 mm posterior to the predicted location of the IOL. This small difference in IOL position will have a tremendous impact on the refractive power of these eyes (McMullen and Gilger, 2006). Given how well they adapt to loss of vision, this may never become a reality but may be worth pursuing in selected cases.

Obtaining manometric intraocular pressure measurements on enucleated rhino eyes and comparing this to applanation and rebound tonometer readings would be ideal in future studies. Detailed anatomic measurement of the lens, to better understand the PACD in pseudophakic eyes after implantation of an IOL could be pursued at the same time, should it ever become possible.

The necessity to examine these animals, in most instances, under field conditions places significant limitations on the type of equipment that can be used and the diversity of measurements that can be obtained. This will certainly affect the accuracy of any data collected. Most of the measurement techniques and methods used in this study could be refined to obtain more accurate data. The study

was not designed to account for differences between species, to establish whether the difference is due to technique or bias would require further, more accurate data collection.

Considering the precarious survival situation rhinos find themselves in, I feel very effort should be made to understand as much about these incredible animals as is possible, and so, hopefully, aid in ensuring their long-term survival for future generations. According to *Montiani-Ferreira et.al (2008)*, “Establishing normal ophthalmic parameters for exotic, wild and laboratory animals is an important scientific area for descriptive vision research” and I feel this work falls within this scope (Montiani-Ferreira et al., 2008a). However, there remains much knowledge to be added to this study.

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ADDENDUMS

Appendix 1: Research Ethics Letter of Approval

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Research Ethics Committee

24 May 2024

LETTER OF APPROVAL

Ethics Reference No	**REC055-21**
Protocol Title	**Documenting prevalence of ocular pathology and establishing biometric parameters in rhinoceroses in South Africa**
Principal Investigator	**Dr JP Burger**
Supervisors	**Dr JD Grewar** **Dr AD Goodhead**

Dear Dr JP Burger,

We are pleased to inform you that your submission conforms to the requirements of the Faculty of Veterinary Sciences Research Ethics committee.

Please note the following about your ethics approval:

1. Please use your reference number (REC055-21) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application (for Post graduate studies e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
2. Note: All FVS animal research applications for ethical clearance will be automatically rerouted to the Animal Ethics committee (AEC) once the applications meet the requirements for FVS ethical clearance. As such, all FVS REC applications for ethical clearance related to human health research will be automatically rerouted to the Health Sciences Research Ethics Committee, and all FVS applications involving a questionnaire will be automatically rerouted to the Humanities Research Ethics Committee. Also take note that, should the study involve questionnaires aimed at UP staff or students, permission must also be obtained from the relevant Dean and the UP Survey Committee. Research may not proceed until all approvals are granted.

We wish you the best with your research.

Yours sincerely

PROF M. OOSTHUIZEN
Chairperson: Research Ethics Committee

100 YEARS OF VETERINARY EDUCATION

Room 0-6, Arnold Theiler Building
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Faculty of Veterinary Science
Fakulteit Veeartsenykunde
Lefapha la Disaense tša Bongakadiruiwa

Appendix 2: Animal Ethics Approval Certificate

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

2 July 2021

Approval Certificate
New Application

AEC Reference No.:	REC055-21
Title:	Documenting prevalence of ocular pathology and establishing biometric parameters in rhinoceroses in South Africa
Researcher:	Dr JP Burger
Student's Supervisor:	Dr AD Goodhead

Dear Dr JP Burger,

The **New Application** as supported by documents received between 2021-05-24 and 2021-07-02 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2021-07-02.

Please note the following about your ethics approval:

1. The use of species is approved:

Species and Samples	Number
Rhino (privately owned)	73

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2022-07-02.
3. Please remember to use your protocol number (REC055-21) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
5. **All incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8434
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Email: marleze.rheeder@up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

We wish you the best with your research.

Yours sincerely

Dr Heike Lutermann
DEPUTY CHAIRMAN: UP-Animal Ethics Committee

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Lefapha la Diseanse tša Bongakadiruiwa

Appendix 3: Ophthalmic examination and data collection sheet

OPHTHALMIC EXAMINATION AND DATA COLLECTION SHEET

CASE NO:		ANIMAL			
Initials of examiner:	JPB	ANIMAL ID			
Examination date		IMMOBILISATION PROTOCOL:			
Name of owner					
Contact number		REASON FOR IMMOBILISATION			
Manager name					
Contact number		SEX		REPRODUCTIVE STATUS:	
		LIFE STAGE/AGE		WILD OR CAPTIVE?	
Location		IF CAPTIVE, BOMA?		IF CAPTIVE, CAMP?	
		HOUSED SINGLY OR GROUP?			
	EXAMINATION FINDINGS				
	OD	OS	COMMENT		
STT					
TONOMETRY					
KERATOMETRY	R1: R2:	R1: R2:			
FLUORESCEIN					
SLIT LAMP	OD				
	OS				
ULTRASOUND	OD				
	OS				
BIOMETRY	OD	AXIAL GLOBE LENGTH		AXIAL LENS THICKNESS	
		ANTERIOR CHAMBER DEPTH		POSTERIOR CHAMBER DEPTH	
	OS	AXIAL GLOBE LENGTH		AXIAL LENS THICKNESS	
		ANTERIOR CHAMBER DEPTH		POSTERIOR CHAMBER DEPTH	
COMMENTS					

JHB & Cape Animal Eye Hospital Ophthalmic Examination Record

Date: Veterinarian:

Client: Client number:

Patient: Patient number: Breed:

History: Sex: Age:

General observations:

	Photopic	Scotopic		R	L		R	L		R	L	
Obstacle course			Menace response			Red light			STT			mm/min
Tracking response			Dazzle reflex			Blue light			Flourescein			
Placing reflex			Palpebral reflex			Direct PLR			Tear BUT			sec
			Corneal reflex			Indirect PLR			IOP			mmHg
									ERG			µV

Gonioscopy: R:
L:

Ultrasound: R:
L:

RIGHT EYE			LEFT EYE
	WNL	ORBIT/GLOBE	WNL
	WNL	EYELIDS	WNL
	WNL	CONJUNCTIVA	WNL
	WNL	THIRD EYELID	WNL
	WNL	SCLERA	WNL
	WNL	NL DUCTS	WNL
	WNL	IRIS	WNL
	WNL	CORNEA	WNL
	WNL	ANT CHAMBER	WNL
	WNL	LENS	WNL
	WNL	VITREOUS	WNL
	WNL	RETINA	WNL

DX OD: DX OS:

RX OD: RX OS

Appendix 4: Client informed consent form and responsible veterinarian consent

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

ANIMAL ETICS COMMITTEE (AEC)

INFORMED CONSENT FORM

We, the undersigned, hereby agree that the animal(s), as specified below, may be used by the researcher(s), as specified below, in the procedures explained below:

1) To be completed by researcher
   - **NAME OF RESEARCHERS(S):**
     Dr JP Burger
     paul@animalevehospital.co.za
   - **NAME OF RESEARCH PROJECT:**
     Ocular biometry and pathology in captive and free-ranging southern white rhinoceros (*Ceratotherium simum)* and south-central black rhinoceros *(Diceros bicornus minor)* in South Africa.
   - **PURPOSE OF RESEARCH PROJECT:**
     There is limited published information describing either population parameters or ocular pathology in rhinoceroses. This study will add to the current body of knowledge and may help identify areas for specific research in future studies.
   - **DETAILED PROCEDURE(S) TO BE PERFORMED**
     Examination of the eyes utilising various modalities is the only reliable means of assessing vision and possible pathology that may or may not be present. As part of a complete ocular exam the following procedures are performed:
     1) Schirmer Tear Test: measures the aqueous component of the precorneal tear film
     2) Tonometry: estimating the intraocular pressure via rebound tonometry
     3) Keratometry: the determination of the curvature of radius of the anterior corneal surface
     4) Slit-lamp biomicroscopy: instrument of choice to examine the anterior ocular segment
     5) Fluorescein dye test: primarily used to identify corneal epithelial ulcerations
     6) Ocular ultrasonography and biometry: ultrasound is a non-invasive imaging modality that allows assessment of the lens, vitreous cavity and retrobulbar space
   - **RISK(S) INVOLVED IN SPECIFIED PROCEDURE:**
     There are no direct risks associated with the ophthalmic examination of the immobilised rhinoceros. All data capture will be opportunistic and performed on animals already undergoing immobilisation for reasons unrelated to this study. Data capture will be abandoned should the physiological state of the immobilised animal warrant immediate recovery and reversal from immobilisation. All immobilisation work will be performed by an experienced wildlife veterinarian, appointed by the owner(s) of the animals identified for immobilisation.
   - **IDENTIFICATION OF ANIMAL TO BE USED:**
     Identification of animals via ear notching, ear tags and/or implanted microchip will be a function of the particular facility where the animals will be examined.
   - **UNMISTAKEABLE DISTINGUISHING DESCRIPTON OF ANIMAL TO BE USED:**
     Rhinoceroses ae large, robust, odd toed ungulates part of the order Perissodactyla and family Rhinocerotidae.

2) To be completed by the animal's owner or person duly authorized to sign on his/her behalf:
   - **NAME OF OWNER**
     _____
   - **HAVE YOU RECEIVED DETAILED INFORMATION REGARDING THE PROPOSED STUDY?**
     _____
   - **HAVE ALL THE RISKS INVOLVED IN THE PROCEDURE BEEN EXPLAINED TO YOU AND DO YOU FULLY UNDERSTAND THESE RISKS?**
     _____
   - **DO YOU GRANT FULL CONSENT FOR THE PROCEDURE TO BE PERFORMED?**
     _____

3) The undersigned parties further agree that no compensation will be payable to the animal's owner or anybody else and that all research associated costs will be covered by the researcher(s).

4) The undersigned parties further agree that this form would serve to fully indemnify the University of Pretoria and the undersigned researcher(s) against any future claims resulting from the specified procedures by or on behalf of the animal's owner

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5) The undersigned parties further agree that no material of any kind including data and research findings, obtained or resulting from the procedure, would be passed on to any third party or used for any purpose other than that specified in this form, except with the written consent of the undersigned owner of the animal.

_____ _____

SIGNATURE RESEARCHER(S) **SIGNATURE OWNER**

_____

SIGNATURE WITNESS

DATE:_____

CONSENT FROM RESPONSIBLE VETERINARIAN

Hereby I, _____ (full names and credentials), working as a qualified veterinarian at _____ (name and street address of practice), consent to administer, maintain and observe the immobilisation of rhinos from _____, while Dr JP Burger performs an ophthalmic examination during the period of the research project. All reasonable care will be taken by me and any follow up treatment and/or emergencies relating to the immobilisation will be my sole responsibility. All immobilisations will be part of the management strategy of _____, and no immobilisation of rhinos will be performed for the sole purpose of this study. Dr JP Burger shall not be held liable for any negligence, wrongful acts and/or omissions done by me or any of my employees.

Signed at _____ (place) on _____ (date and time)

_____

Dr

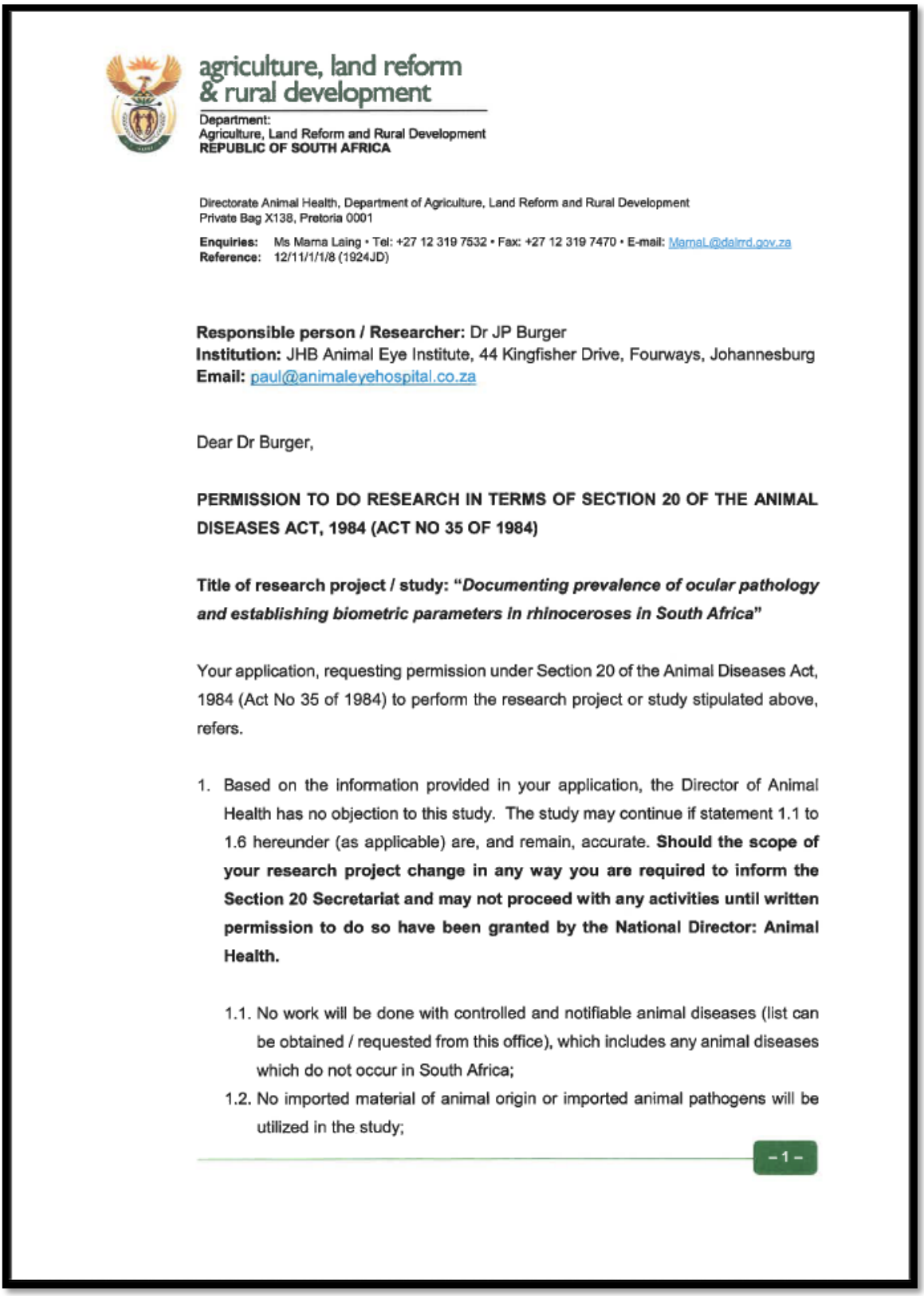
_____

JP Burger (Primary researcher)

_____

Witness

Appendix 5: Department: Agriculture, Land Reform and Rural Development; Section 20 permit



agriculture, land reform & rural development

Department:
Agriculture, Land Reform and Rural Development
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Land Reform and Rural Development
Private Bag X138, Pretoria 0001

Enquiries: Ms Mama Laing • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: MamaL@dalrrd.gov.za
Reference: 12/11/1/1/8 (1924JD)

Responsible person / Researcher: Dr JP Burger
Institution: JHB Animal Eye Institute, 44 Kingfisher Drive, Fourways, Johannesburg
Email: paul@animaleyehospital.co.za

Dear Dr Burger,

PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO 35 OF 1984)

Title of research project / study: "*Documenting prevalence of ocular pathology and establishing biometric parameters in rhinoceroses in South Africa*"

Your application, requesting permission under Section 20 of the Animal Diseases Act, 1984 (Act No 35 of 1984) to perform the research project or study stipulated above, refers.

1. Based on the information provided in your application, the Director of Animal Health has no objection to this study. The study may continue if statement 1.1 to 1.6 hereunder (as applicable) are, and remain, accurate. **Should the scope of your research project change in any way you are required to inform the Section 20 Secretariat and may not proceed with any activities until written permission to do so have been granted by the National Director: Animal Health.**

   1.1. No work will be done with controlled and notifiable animal diseases (list can be obtained / requested from this office), which includes any animal diseases which do not occur in South Africa;
   1.2. No imported material of animal origin or imported animal pathogens will be utilized in the study;

– 1 –

1.3. No samples that originate from a biobank will be used in the study;
1.4. No clinical studies will be performed in the target species, either in a laboratory or in the field;
1.5. The areas where the samples are to be collected are not under restriction for controlled or notifiable diseases to which the species of animal, from which the samples are obtained, is susceptible;
1.6. No samples or products that have not been passed as fit for human consumption will be obtained from an abattoir.

2. In addition to the conditions mentioned in point 1, you are responsible for ensuring that your research project or study complies with all or part of the following, **as applicable**:

2.1. Permission to perform research under Section 20 of the Animal Diseases Act, 1984 (Act no 35 of 1984) does not relieve the researcher of any responsibility which may be placed on him/her by any other Act of the Republic of South Africa, including the Veterinary and Para-Veterinary Professions Act, 1982 (Act No 19 of 1982), the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act No 36 of 1947), the Medicines and Related Substances Control Act, 1965 (Act No 101 of 1965), the Genetically Modified Organisms Act, 1997 (Act No 15 of 1997) and the National Environmental Management: Biodiversity Act, 2004 (Act No 10 of 2004);
2.2. No part of the study may begin until valid ethical approval has been obtained in writing from the relevant South African authority;
2.3. All biological or potentially infectious material must be packaged and transported in accordance with International Air Transport Association (IATA) requirements and the National Road Traffic Act, 1996 (Act No. 93 of 1996);
2.4. Any incidence or suspected incidence of a controlled or notifiable disease in terms of the Animal Diseases Act, 1984 (Act No 35 of 1984), must be reported immediately to the responsible state veterinarian;
2.5. Wild and captive rhinoceros as part of planned rhinoceros immobilisation operations may be used for this study;
2.6. No samples will be taken during the examinations and no animals will be transported to or from the capture area for the purpose of this study;
2.7. Samples or material may not be outsourced or used for further/other research without prior written approval from the Director of Animal Health;

2.8. All potentially infectious material utilised or generated during or by the study is to be destroyed at completion of the study and only a registered waste disposal company may be used for the removal of waste generated during or by the study;

2.9. Records must be kept for five years for auditing purposes.

Written permission from the Director of Animal Health must be obtained prior to any deviation from the conditions. Application must be sent in writing to MarnaL@dalrrd.gov.za.

Failure to obtain written permission as above may be considered a contravention of the Animal Diseases Act, 1984 (Act No 35 of 1984).

Expiry date of this permit: 31 December 2023

Kind regards,

Dr Mpho Maja
DIRECTOR: ANIMAL HEALTH
Date:
2021-04-29

SUBJECT: Permission to do research in terms of Section 20 of the Animal Diseases Act, 1984 (Act No 35 of 1984)

Appendix 6: Presentations and publications arising from this study

Possible journals for publication of research:

1) Veterinary Ophthalmology
2) Journal of Zoo and Wildlife Medicine

Possible congresses for presentation of research

1) ECVO Congress 2025
2) SAVA Congress 2025