Ocular pathology in Warmblood horses (*Equus caballus*) in South Africa

by

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FOR

RAYMOND

(1947-2006)

Thank you for teaching me the

important things in life

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Animal Ethics Committee

PROJECT TITLE	**Prevalence of ocular pathology in warmblood horses *(Equus caballus)* in South Africa**
PROJECT NUMBER	**V068-17**
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NUMBER OF SAMPLES	**554**	
Approval period to use animals for research/testing purposes		**June 2017- June 2018**
SUPERVISOR	**Dr. A Goodhead**	

KINDLY NOTE:

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

APPROVED	Date 26 June 2017
CHAIRMAN: UP Animal Ethics Committee	Signature

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LIST OF ABBREVIATIONS

%	Percentage
cPLR	Consensual pupillary reflex
dPLR	Direct pupillary reflex
EHV	Equine herpes virus
ERU	Equine Recurrent Uveitis
IOP	Intraocular pressure
MHC	Major histocompatibility complex
mm	Millimetres
mmHg	Millimetres mercury
mm/min	Millimetres per minute
N	Number of animals
n.d.	No date
NLD	Nasolacrimal duct
ONH	Optic nerve head
P	Estimation of statistical significance
PPM	Persistent pupillary membrane
PTF	Precorneal tear film
RPE	Retinal pigment epithelium
SCC	Squamous cell carcinoma
SD	Standard deviation
SNP	Single nucleotide polymorphism
STT	Schirmer tear test
yr	Year

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SUMMARY

Objective: To determine the prevalence of ocular abnormalities in Warmblood horses (*Equus caballus*) in South Africa.

Background: Warmblood horses have become one of the most popular horse breeds around the world, as well as in South Africa, and are commonly utilised for the equestrian sports of show jumping, dressage and eventing. The presence of vision-threatening abnormalities in such horses could have severe consequences. This prompted the current investigation into assessment of ocular abnormalities in Warmblood horses, which have no history of previous vision problems.

Design: Descriptive, observational study

Materials: One hundred and four Warmblood horses around various locations in the Gauteng and North West Provinces of South Africa.

Methods: Ophthalmic examination was performed using Schirmer tear test (STT) examination, tonometry, fluorescein dye testing, slit lamp biomicroscopy and direct ophthalmoscopy. Pupils were dilated in all horses for comprehensive examination of the fundus. Age, gender, Warmblood type, coat colour, date and time of examination, utility and housing type were all recorded at time of examination.

Results: Age range was 0.4-30 years (mean 10.69 years, median 9 years); with 8 stallions (7.7%), 33 mares (31.7%) and 63 geldings (60.6%). Age was categorised into 4 quartiles namely, 0-6 year old, 7-9 year old, 10-15 year old and 15+ years olds. STT results were 24.4mm/min for the right eye and 25.0mm/min for the left eye, and there was no statistical difference between eyes, genders or age categories. Intraocular pressure (IOP) for the right eye was 36.5mmHg and for the left eye was 32.6mmHg, and there was no statistical difference between eyes, gender or age categories. Cataracts were seen in 19 eyes (9.1%) and in 13 horses (12.5%). The presence of cataracts increased with age, however was only statistically significant in horses older than 15 years old. Chorioretinal lesions were seen in 100 eyes (48.1%) and in 65 horses (62.5%). Of the 100 eyes affected, 7 eyes had peripapillary 'butterfly' chorioretinal lesions (3.4%), 14 eyes had non-peripapillary chorioretinal lesions (6.7%), 88 eyes had focal ‘bullet hole’ chorioretinal lesions (42.3%). The presence of chorioretinal lesions increased with age, however was only statistically significant in horses aged 7-9 years old and 15+ years old. Embryological remnants were detected in 140 eyes - anterior lens capsule opacity 1 (0.5%) and iris to iris persistent pupillary membranes (PPM) 139 (67.3%). Other findings included iris hyperpigmentation 16 eyes (7.7%), conjunctivitis 12 eyes (5.8%), vitreal degeneration 8 eyes (3.9%), asteroid hyalosis 4 eyes (1.9%), uveal cyst 3 eyes (1.4%), linear chorioretinal scar 3 eyes (1.4%), eyelid scar 2 eyes (1%), eyelid mass 2 eyes (1%), prominent conjunctival follicles 2 eyes (1%), conjunctival mass 2 eyes (1%), corneal fibrosis

2 eyes (1%), linear keratopathy 1 eye (0.5%), corpora nigra hyperplasia 1 eye (0.5%), torn corpora nigra 1 eye (0.5%), posterior synechiae 1 eye (0.5%), vitreal herniation 1 eye (0.5%), chalazion 1 eye (0.5%) and an optic nerve mass 1 eye (0.5%).

Conclusions and Clinical Relevance: Chorioretinopathies and cataract formation were the most common lesions observed in Warmblood horses in South Africa. Such lesions have the ability to result in significant visual impairment and even blindness. This reiterates the importance of ocular examination as a part of routine health checks, as well as during pre-purchase examinations, especially in horses utilised for equestrian sports.

Keywords: equine, horse, Warmblood, prevalence, surgery, chorioretinopathy, cataract, eye, eye disease, lens, retina

Chapter 1

INTRODUCTION

The modern horse, *Equus caballus*, is a part of the family Equidae and over 300 breeds and types of horse exist nowadays (‘Horse’, 2020). Man has greatly influenced the development of horses by selecting them for the specific types of work carried out. A gross generalisation historically used assigned horses into categories termed “Coldblood” and “Warmblood” (‘Warmblood’, 2020). The former being the heavier draught horses believed to have come from the northern parts of Europe, whereas Warmblood refers to the medium weight types originally used for transport, power and war purposes. Historically, it was only after the First World War that Warmbloods were being used for competitive sporting purposes such as eventing, dressage and showjumping, which they are now specifically bred for (‘The South Africa Warmblood Horse’, (n.d.).

Fig. 1. A Warmblood horse (Grissel, Theunis. “Show jumping competition.” 2019. jpg)

The first Warmblood horses imported into South Africa were from Namibia, which had a high proportion of Hanoverian genetics ('The South Africa Warmblood Horse', (n.d.). These animals were crossed with Warmbloods from other parts of Europe, as well as Thoroughbreds, to form the South African Warmblood which we know today. Form and temperament are essential to these disciplines and are highly selected for.

Warmblood horses commonly encountered in Europe are from Denmark, Sweden, Germany, Belgium and Holland. Warmbloods born in South Africa are termed South African Warmbloods and can be registered with the South African Warmblood Horse Society. There are over 7000 Warmbloods registered with the society today.

The South African Equestrian Federation (SAEF) represents equestrian sport in South Africa and is a local representation of the Fédération Equestre Internationale (FEI). Within the SAEF there are various disciplines such as show jumping, dressage and eventing.

Today our equine industry in South African is expanding at a rapid rate and the Kyalami area of Gauteng, situated between Johannesburg and Pretoria is a hub of equestrian activities. This area is said to have the highest equine concentration in the Southern hemisphere.

When breeding Warmblood horses, little emphasis is placed on ophthalmic examination, as conformation, appearance and movement play a much larger role in the selection criteria.

To the author's knowledge no study on general ophthalmic examination of the Warmblood horse exists, in contrast to other breeds such as the Thoroughbred (Hurn & Turner 2006), Lipizzaner (Rushton *et al*., 2013a), Exmoor Pony (Pinard *et al*., 2011), Old Kladruber Horses (Andrysikova *et al., 2019)*, Polish Arabian horses (Paschalis-Trela *et al*., 2017) , Rocky Mountain Horses (Grahn *et al*., 2008; Ramsey *et al*., 1999) and miniature horses (Plummer & Ramsey 2011). Additional studies exist involving specific groups of horses such as foals (Barsotti *et al*., 2013; Labelle *et al*., 2011; Latimer *et al*., 1983; Turner 2004), geriatric horses and ponies (Chandler *et al*., 2003; Ireland *et al*., 2012), blue-eyed horses (Bërgstrom *et al*., 2014), working horses (Scantlebury *et al*., 2014; Starkey *et al*., 2014; Thangadurai *et al*., 2010) and by riding club (Bazargani *et al*., 2011; Taghipour Bazargani *et al*., 2011). Reports on congenital ocular abnormalities exist for specific breeds such as Rocky and Kentucky Mountain horses (Grahn *et al*., 2008; Ramsey *et al*., 1999).

Objectives of this study were to determine the prevalence of ophthalmic pathology, to determine if there is an age-related correlation and note any normal anatomical variations. Further to this we may advise and encourage regular ophthalmic examination of all horses used in a competitive setting, especially horses which may be used for breeding purposes, as certain ocular conditions are thought to have a heritability factor (Kulbrock *et al*., 2013).

Chapter 2

LITERATURE REVIEW

Equine vision is a complex subject and veterinary ophthalmologists are often confronted with questions varying from what exactly horses see, their visual acuity and how to deal with a blind horse. A comprehensive understanding is often needed as horses are used for various functions. In South Africa, Warmblood horses are commonly used in disciplines such as show jumping, dressage and eventing.

There are numerous ocular disorders described in horses, with some being exclusive to this species. The effect of such conditions ranges from minimal to profound, however severity does not always positively correlate with poor vision, as impairment of vision in one eye can be masked by normal vision in the contralateral eye (Miller & Murphy, 2011). This is demonstrated by many one-eyed horses having competed at a top level with great success. Famous one-eyed Warmblood horse examples include Tornesch ridden by Marlin Bayard (show jumper), Adventure de Kannan Hickstead ridden by Trevor Breen (eventing) and Cornado NRW ridden by Marcus Ehning (show jumper).

Safety concerns should be raised in horses with visual defects, as certain ocular conditions may result in decreased functional ability, thereby affecting the performance and safety of both horse and rider. An example of such would be a horse rearing for no obvious reason or stopping abruptly and refusing to jump (Miller & Murphy, 2011).

The orbits of equine eyes are large and positioned laterally. This allows for a wider field of view. Horses have 190-195^{o} and 350^{o}, monocular and binocular horizontal visual fields, respectively (Gilger 2013). Their vertical visual field is up to 178^{o} in the vertical axis (Gilger 2013). However, the visual field can change depending on the head position. Horses therefore have near complete vision around the axis of the head with the exception of a few blind spots. These blind spots are located directly above and perpendicular to the forehead, behind the head, beneath the nasal area, as well as in the superior visual field where light falls on the optic nerve head (Miller & Murphy 2011). In a large study in the U.K. involving both horses and ponies, 83.6% of horses and ponies were considered emmetropic (Bracun *et al*., 2014), meaning they were not afflicted by any refractive errors.

Equine eyes have large corneas and have a horizontal, ovoid pupil shape. This is thought to allow for increased amounts of light to enter the eye, as well as a horizontal field of vision, which is essential in prey species (Miller & Murphy, 2011).

With regards to the fundus, like many terrestrial species, the horse is in possession of a tapetum lucidum, which is fibrous in nature (Wilke, 2011). This shiny, reflective structure of the choroid allows for light entering the eye to first stimulate the photoreceptor layer, which subsequently is reflected off the tapetum, thereby restimulating the photoreceptor layer a second time. This enhanced light capturing ability allows animals better vision in dim or darker conditions (Miller & Murphy, 2011).

Horses appear to be equally well adapted to photopic conditions, with both the dorsal and ventral pupil margins being equipped with corpora nigra, which are structures which may reduce glare from the sun and reduce back scatter of light off the ground, respectively (Miller & Murphy 2011). Additionally, the equine lens containing a yellow pigment, which filters out blue light (Murphy *et al*., 1993). Blue light is scattered more easily than red light, and therefore filtering reduces the backscatter and glare (Miller, 2003).

The nasal retinal portion has a greater vertical width than the corresponding temporal retina, which then increases the temporal visual field (Miller & Murphy 2005). Horses have dichromatic colour vision with two cone types with a peak sensitivity of 428-429 nm (blue or short cone) and 539-545 nm (middle to long wavelength – sensitive cone) (Miller & Murphy 2011). Dorsal to the optic disc is an area known as the visual streak. The temporal region of the streak has increased density of retinal ganglion cells and photoreceptors, thereby providing the area of highest visual acuity (Guo *et al*. 2000; Guo *et al*. 2002).

Comprehensive ophthalmic examinations should accompany any history of behavioural issues during competition and routinely in a pre-purchase examination. Problems may then be identified and possibly corrected or prevented from progressing. Early detection of ocular disease may improve the prognosis and outcome.

2.1 Ophthalmic examination

Examination of the globe and adnexa is an essential part of assessing vision and documenting ocular pathology.

2.1.1 Schirmer Tear Test

The precorneal tear film (PTF) is an essential component of the eye, as it contributes to the health of the ocular surface and optical clarity. The PTF is composed of three indistinct layers, namely the lipid, aqueous and mucin layers.

A Schirmer tear test (STT) test is primarily carried out to quantify the production of the aqueous component in the tear film and establish baseline tear production values for the individual animal (Featherstone & Heinrich 2013). The STT should be performed at the beginning of the ophthalmic examination as any manipulation of the globe, cornea and conjunctiva results in reflex tearing and therefore will result in falsely elevated values (Featherstone & Heinrich 2013). The STT 1 is used to measure basal and reflex tearing without the use of a topical anaesthesia, whereas the STT 2 measures only basal tearing with the use of topical anaesthesia (Featherstone & Heinrich 2013). The latter is performed to rule out reflex tearing. This test is carried out by placing a 5 X 35 mm Whatman filter paper no. 41 into the lower fornix of the eye (Schirmer Tear Test, Merck Animal Health). The strip is initially folded 5mm from the lower end, placed in the fornix for 1 minute and the amount of tear production is indicated by the progression of the blue dye impregnated along the strip. A value is recorded in mm/min. Values should be read immediately otherwise falsely elevated values may be observed due to the fluid continuing to run on the strip via wick action. Conversely, if read at a later time, fluid may have evaporated from the strip causing an underestimated value. Results of STT values in healthy horses free of ocular disease are varied but, have been documented between 11-30mm/min, as well as 15-20mm/30 sec (Marts *et al*., 1977; Moore, 1992). A single drop of 1% tropicamide has been shown to have statistically significant reduction in STT values in clinically normal horses (Ghaffari *et al*., 2009). Beech *et al*. (2003) concluded that neither signalment, housing, season nor location showed a significant effect on tear production and it was shown that there is a large variation in STT values in horses and ponies. Variations of 10mm/min to greater than 35mm/min were noted. STT-1 values of less than 10mm establish a diagnosis of keratoconjunctivitis sicca (KCS) (Crispin, 2000).

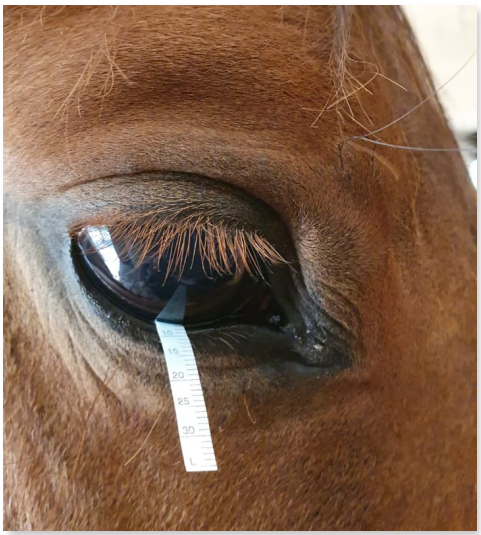

Fig. 2. Quantitive assessment of the tear film using a Schirmer tear test

2.1.2 Intraocular pressure

Tonometry is the indirect measure of IOP and should be a routine part of any ocular examination (Featherstone and Heinrich, 2013). This is essential in globes exhibiting pain, hyperaemia or congestion, corneal oedema, trauma, lens instability, exophthalmos or in patients in which the contralateral globe has been afflicted with glaucoma (Plummer *et al*., 2013).

There are two main methods of measuring IOP namely digital or instrumental tonometry. Instrumental tonometry involves indentation tonometry, applanation tonometry and rebound tonometry. Only the latter two are reliable in the field of veterinary ophthalmology (Gelatt 1994).

Applanation tonometry consists of a 2.36mm contact probe, which connects with the corneal surface. The force required to flatten the cornea is converted into an IOP reading. A commonly used application tonometer is the Tono-Pen (Reichert, Buffalo, NY). Topical anaesthesia is required for usage, due to direct contact with the cornea.

IOP measurements in horses using applanation tonometers, Mackay-Marg and the Tono-Pen, resulted in average readings of 23.5mmHg and 23.3mmHg, respectively (Miller *et al*. 1990).

If the intraocular pressure is very low, it can be difficult to obtain a reading. While it has been shown that auriculopalpebral nerve blocks do not alter IOP readings in normal horses, severe blepharospasm may increase intraocular pressure (Van der Woerdt *et al*., 1995).

Rebound tonometry makes use of a magnetic field and a magnetised probe that is propelled onto the cornea at a set distance. The rate at which the probe decelerates off the cornea is then translated into an IOP value. The more turgid the globe is, the more rapid the deceleration and the higher the IOP reading.

A commonly used rebound tonometer in veterinary ophthalmology is the Tono-Vet apparatus (ICare, Helsinki, Finland), which provides accurate values simply, quickly and easily. The procedure is noninvasive, does not require topical anaesthesia and causes minimal discomfort to the animal. This instrument has two different measuring settings, one for dogs and cats, and one for horses. The Tono-Vet records six measurements, which are then averaged to provide a final measurement.

Higher than normal values establish a diagnosis of glaucoma. Any animal with a red eye should have IOP readings performed on both eyes (Plummer *et al*., 2013). However, any good ophthalmic examination should be accompanied by IOP readings as subclinical abnormalities can be detected earlier. Alternatively if a low reading is detected, an underlying uveitis should be considered (Hendrix, 2013).

Normal values for IOP in conscious and unsedated horses using rebound tonometry with a Tono-Vet apparatus were 22.1 ± 5.9 mm Hg (range, 10 to 34 mm Hg) (Knollinger *et al*., 2005).

Numerous factors can influence IOP readings such as type of tonometer, user's technique, use of various types of anaesthesia (e.g. topical anaesthetic, sedation and regional nerve blocks), physical restraint methods, various disease processes and time of day. Van der Woerdt *et al*. (1995) demonstrated that using xylazine, as a sedative, can affect the IOP by up to 23%. Marzok *et al*. (2014) showed a significant reduction in IOP using intravenous romifidine as a sedative. Interestingly, Holve (2012) saw a significant increase in IOP in patients treated with topical anaesthesia versus an untreated group. The effect of tropicamide on IOP has not been studied in horses, but has been shown to affect IOP readings in other species such as cats (Gomes *et al*., 2011). Circadian rhythm has been shown to affect IOP readings in the horse. IOP was found to be lowest during a dark phase and highest towards the end of the light phase (Bertolucci *et al*., 2009). It is thought that eyelid tension may result in falsely elevated IOP readings, however in two studies this has been disproven (Miller *et al*., 1990; Van der Woerdt *et al*., 1995). Komáromy *et al.* (2006) showed head position to have a statistically significant on the IOP of horses when comparing values with the head above and level the heart level.

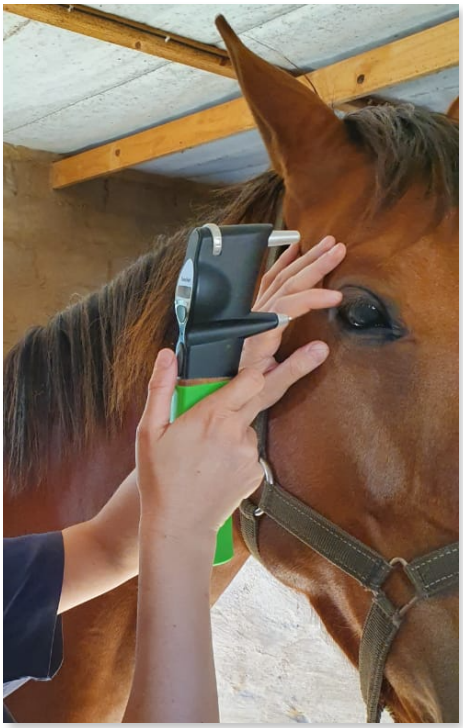

Fig. 3. Rebound Tonometry to measure intraocular pressure

2.1.3 Fluorescein Dye Test

Sodium fluorescein is an orange, water soluble, weakly acidic dye which peaks at a 490nm absorption spectrum (Featherstone & Heinrich 2013).. When placed in an alkaline environment, fluorescent green light is emitted with a peak wave length at 520nm (Featherstone & Heinrich 2013).. Fluorescein-impregnated strips of paper (Haag-Streit Diagnostics, Bern, Switzerland) are lightly applied to the dorsal bulbar conjunctiva. Closure of the eyelids distributes the fluorescein stain across the cornea. Due to the hydrophilic and lyophobic nature of fluorescein, lipid-containing structures such as the corneal and conjunctival epithelium repel fluorescein (Featherstone & Heinrich 2013). However, should such structures be damaged, the fluorescein is absorbed by the corneal or conjunctival stroma leading to a green fluorescence, indicating an ulcer. The main purpose for the use of the fluorescein dye test is to detect corneal ulcers, however other common uses include identification of conjunctival epithelial defects, aqueous humour leakage from a full thickness corneal wound (Seidel Test), descemetocoele diagnosis, patency of the nasolacrimal ducts (NLD) (Jones Test) and qualitative tear film deficiencies (tear film breakup time) (Featherstone & Heinrich 2013).

2.1.4 Tropicamide application

Tropicamide (Mydriacyl; Alcon, Forth Worth, U.S.A.) is an antimuscarinic drug used to cause cycloplegia of the ciliary body, and mydriasis of the iris (Herring, 2013). Application should only be performed after examination of the anterior segment, tonometry, direct pupillary light reflex (dPLR), consensual pupillary light reflex (cPLR) and the menace response have been assessed (Featherstone & Heinrich 2013). Pupil dilation takes approximately 15-30 minutes and facilitates examination of the posterior segment of the eye, namely the lens, vitreous and fundus (Herring 2013). Mydriacyl has a relatively short acting effect but has been shown to last up to 12 hours in horses with a maximum effect on dilation at 5 hours (Gelatt *et al*., 1995). Should uveitis or synechiae be present this may hamper complete dilation of the pupil and should be noted as such.

2.1.5 Slit lamp biomicroscopy

Slit lamp biomicroscopy allows for a thorough examination of the anterior eye segment, namely the adnexa, cornea, anterior chamber, iris, lens as well as the anterior vitreous. Veterinary slit lamp biomicroscopes are handheld and are a combination of a binocular microscope and light source, which, in most models, pivot around a fixed microscope (Featherstone & Heinrich 2013). The use of a slit lamp biomicroscope allows for excellent three-dimensional magnification (5-40X depending on the model) with various light source forms (e.g. light-emitting diode, halogen or xenon) (Featherstone & Heinrich 2013). Light shape, intensity, beam width and length, orientation and colour can all be adjusted. The focal light allows for evaluation of an optical section. Additionally,

the slit beam is used to asses any topographical variations in the tissues such as tumours or corneal ulcers (Featherstone & Heinrich 2013). Most slit lamps come with three different colour filters, namely cobalt blue, red-free (green) and neutral density (Featherstone & Heinrich 2013). The instrument is handheld, is placed at 7-10cm focal distance, whilst angling the light source 20-45 degrees from the microscope's axis, and one can alternate the light beam from different sides to best visualise structures (Featherstone and Heinrich, 2013).

Fig. 4. A Keeler slit lamp biomicroscope (Keeler, 2019)

2.1.6 Direct ophthalmoscopy

Basic direct ophthalmoscopy makes use of a direct ophthalmoscope, which consists of coaxial optics, as well as power and light source (Featherstone & Heinrich 2013). The fundus can be examined using direct or indirect ophthalmoscopy, however in equine patients direct ophthalmoscopy is more practical. Once the pupil is dilated a larger view of the fundus is visible (Stoppini & Gilger 2016). Distant direct ophthalmoscopy with the patient positioned at an arm's length away gives a good general overview of the globe (Featherstone & Heinrich 2013). Opacities in the visual axis are easily seen when using the tapetal reflection as a background (retroillumination) (Featherstone & Heinrich 2013). These opacities are seen as black spots, as they block the light reflecting off the fundus. Such areas can then be investigated further with a slit-lamp biomicroscope.

Direct ophthalmoscopy utilises the refractive power of both the examiner and that of the patient, namely the cornea and lens. The majority of fundi are in focus between 0-2 diopters provided the examiner is emmetropic (Featherstone & Heinrich 2013). Factors such as globe size and working distance affect magnification and therefore it is important to compare lesion size with that of the

optic nerve size diameter (Featherstone & Heinrich 2013). The fundus image is real, upright and magnified to various degrees, dependent on the species.

Due to the large size of equine eyes, the slit lamp light source is excellent for direct ophthalmoscopy. Examination of the posterior segment is performed by direct focal illumination (i.e. transillumination). The instrument's light source is placed adjacent to the examiner's head with the light directed into the horse's eye. It is best to stay in contact with the horse by placing one's hand on the horse's head. This avoids trauma by letting the examiner know how the horse is moving during the examination.

Fig. 5. Examination using a slit lamp biomicroscope

2.2 Ophthalmic disorders in horses

Several studies exist describing common ocular diseases and their pathogenesis in horses., but studies demonstrating the prevalence of equine ocular disease, especially in specific breeds, are few.

Two studies involving neonatal foals demonstrated ocular abnormalities in both healthy and ill individuals (Barsotti *et al*., 2013) and (Labelle *et al*., 2011). Most of the findings in the group of healthy Standardbred foals were suspected to result from parturition such as retinal and subconjunctival haemorrhages. At one and two week follow up periods, all retinal and subconjunctival lesions had resolved. Data from the systemically ill foals showed 55.7% had 1 or more ophthalmic lesions, and of those affected, 61.5% had potentially vision threatening lesions. Latimer *et al*. (1983) demonstrated a low incidence of congenital ocular disease in the horse.

A retrospective study of 2846 racing thoroughbreds in Japan with ulcerative keratitis showed that racing was the cause of the ulceration in 64.3% of these horses (Wada *et al*., 2010). Horses which presented for examination the day after racing had a faster healing time compared to those that presented at a later stage. Another study involving racing Thoroughbreds in Australia observed ophthalmic findings in 67.6% of horses, of which 7.5% were considered potentially vision threatening defects (Hurn & Turner 2006). Interestingly, the latter horses had no known history of visual impairment, however, when investigated further, a handful of these cases did indeed have a history of trauma or behavioural issues.

A survey of Lipizzaners stabled at different locations reported that 76% of examined horses had ocular findings. These were predominantly associated with the conjunctiva, cornea or fundus (Rushton *et al*., 2013a).

A more recent study of 615 Polish Arabian horses found that 9.8% of the population had ocular disease. Of the abnormalities noted, lesions related to Equine Recurrent Uveitis (ERU) had the highest prevalence at 5.5% (Paschalis-Trela *et al*., 2017).

Dywer (2017), using data gathered over a 6 year time period from 5790 general practice equine patients, showed that 11.5% of these horses had some degree of ocular pathology. The most common ocular pathologies noted in these patients were corneal ulceration, unilateral eyelid swellings, blind eye(s), uveitis, conjunctivitis and significant cataracts.

A cross sectional study in which 200 geriatric horses aged 15 years or older were assessed, showed vitreal degeneration, cataracts and senile retinopathy as the most common ocular abnormalities present. The latter two conditions were present in significantly older animals than in horses without such lesions (Ireland *et al*., 2012).

Ocular abnormalities in Miniature horses were observed in individuals with a flaxen or white mane and tail colour and a chocolate coat colour (Plummer & Ramsey 2011). This finding was similar to the multiple congenital and hereditary ocular abnormalities in Rocky Mountain Horses (Grahn *et al*., 2008; Ramsey *et al*., 1999).

2.2.1 Eyelids

The eyelids of horses are similar to those of other domestic species and consist of haired skin, connective tissue, muscle, a tarsal plate and the palpebral conjunctiva. The eyelids play an exceptionally important role, not only to protect the globe, but also to contribute to the tear film, distribute the tear film over the ocular surface, remove foreign particles and regulate the amount of light entering the eye.

Eyelid neoplasia

Eyelid neoplasia is fairly common in equine patients and accounts for approximately 10% of ocular neoplasia (Baker & Leyland 1975; Sundberg *et al*., 1977) with squamous cell carcinoma (SCC), papilloma, melanoma and lymphosarcoma constituting the majority of cases. A biopsy and histopathology is required to confirm a diagnosis and early surgical excision is advised.

Eyelid trauma and fibrosis

In modern day society, many horses are stabled in close confinement and are subject to frequent travel for various sporting events. Commonly offending objects, which may cause trauma, include the sharp edged objects such as building structures and nails. Given the prominent location and lateral position of horses eyes, together with their utility and environment, ocular trauma is not uncommon (Giuliano 2011). Eyelid lacerations are usually obvious, however severity of the lesion and outcome do not always correlate well (Giuliano 2011). Surgical intervention is usually needed as retraction of an unrepaired wound can result in fibrosis and eyelid retraction which may affect the blink reflex and adequate eyelid closure. Healing is usually rapid due to the excellent blood supply of the eyelids, provided infection is not present (Giuliano 2011). Additionally, assessment of the facial nerve should be evaluated in all cases of eyelid trauma. The auriculopalpebral branch of the facial nerve supplies motor innervation to the eyelids, runs superficially and therefore has the ability to be easily damaged (Gilger and Stoppini 2005).

2.2.2 Conjunctiva

The conjunctiva surrounds both the globe and the inner eyelid and is termed the bulbar and palpebral conjunctiva respectively. Due to the dense blood supply to the conjunctiva, it has the ability to heal rapidly, however also becomes rapidly inflamed in response to irritation (Giuliano 2011).

Conjunctivitis

Conjunctivitis denotes inflammation of the conjunctiva and clinical signs include hyperaemia and chemosis with or without a serous or mucoid ocular discharge. Conjunctivitis can be classified as primary or secondary, the latter being subsequent to conditions such as corneal disease and uveitis (Giuliano 2011). Primary conjunctivitis is not uncommon in horses due to the dusty environment in which they are housed. This combined with local weather conditions such as wind, can be severely irritating to the conjunctiva. Occasionally conjunctival follicles can be seen which are indicative of an underlying immune-mediated pathology (Giuliano 2011).

Conjunctival Neoplasia

SCC is the most common neoplasia seen in the equine eye and adnexal structures (Lavach & Severin 1977; Strafuss, 1976). Commonly affected structures include the bulbar conjunctiva, corneoconjunctiva, third eyelid and eyelids. Various factors are thought to play a role in the transformation into a SCC such as increased longitude, altitude or mean annual solar radiation (Dugan *et al*., 1991). The same paper reported an increased prevalence with decreased latitude. As one would expect, prevalence appears to increase with age and the Appaloosa breed was shown to be overrepresented when compared with draught and quarter horses (Dugan *et al*. 1991). Stallions and mares were 5 and 2 times respectively less likely to develop SCC in comparison to geldings (Dugan *et al*., 1991).

2.2.3 Cornea

Linear keratopathy

Linear keratopathy is defined as a non-ulcerative, non-painful keratopathy, the aetiology of which is unknown (Brooks & Matthews 2007). These lesions are seen as well defined, non-oedematous, parallel striae, which are situated at the level of Descemet's membrane (Brooks & Matthews 2007). They are often single, 1-2mm in width, do not bridge the entire width or height of the cornea, and are more commonly seen in the horizontal plane. The lesion is created by a thinning of Descemet's membrane with a normal adjacent endothelial layer (Brooks & Matthews 2007). A differential diagnosis for such lesions would include glaucoma, however this condition usually is associated with multiple branching striae, corneal oedema and ultimately corneal fibrosis. A further differential would be larval migratory tracts.

A study by Rushton *et al*. (2013b) in Lipizzaners showed linear keratopathy to be non-progressive over an 18 month period. This finding is not thought to be congenital, however the prevalence of which was found to increase with progressive age.

Rushton *et al*., (2013a) found 2 out of 203 Lipizzaners examined had a linear keratopathy. The prevalence of linear keratopathy in other studies was also shown to be low at 0.04% in a study of Old Kladruber Horses (Andrysikova *et al*., 2018).

2.2.4 Uvea

The uveal tract is composed of the iris, ciliary body and the choroid. The iris constricts or dilates according to the ambient light and to allow the entry of varying degrees of light as needed for visual acuity. The ciliary body is divided into two components, namely the *pars plicata* and *pars plana*.

The *pars plicata* consists of the ciliary processes, which are responsible for the formation of aqueous humour, as well as having a role in lens stabilisation and accommodation. In the horse, the latter occurs by insertion of lens zonules onto both the ciliary processes and the intervening valleys. Both of these fibre types continue onto the *pars plana*, and additional fibres connect the apices of the processes laterally.

The choroid is the most posterior portion of the uvea and is composed of mainly capillaries and larger blood vessels. This layer provides the retina with its main supply. The equine tapetum is fibrous and is situated on the dorsal half of the fundus. This structure allows for restimulation of the photoreceptor layer as incoming light gets reflected off.

Uveal cysts

Uveal cysts have been noted in horses of various ages, can be uni- or bilateral and are not associated with pain or discomfort. Some cysts are an incidental finding on examination, however some are the primary complaint, as they can enlarge and physically hamper vision by occluding the visual axis. Cysts are generally smooth and round to oval in shape, are a fluid filled extension of the posterior iris epithelium and the inciting cause is yet to be determined, however cysts anecdotally appear to be present in middle aged to old horses and unrelated to inflammation (Dziezyc *et al*. 1990). It is postulated that cyst formation is related to failure of the two layers of the neuroectoderm, thereby allowing fluid to accumulate between the two-layered posterior iris (Gilger & Hollingsworth 2016).

There are four different locations for uveal cysts, namely free floating in the anterior chamber, the pupil margin, which may be associated with the corpora nigra, ciliary body or iris stroma. An important differential diagnosis for an attached uveal cyst would be an iridial tumour, which can be differentiated using ocular ultrasound, however cysts generally appear clear when transilluminated.

Rocky Mountain Horses, Miniature Horses and ponies have all been reported to have a higher incidence of uveal cysts. This is thought to be related to the equine Multiple Congenital Ocular Anomalies syndrome (Komáromy *et al*., 2011; Plummer & Ramsey 2011; Ramsey *et al*., 1999).

Anterior segment dysgenesis

Persistent pupillary membranes are the common form of anterior segment dysgenesis.

Persistent pupillary membranes

During normal embryogenesis, the hyaloid system forms the primitive vascular supply to the developing lens. The hyaloid artery enters the eye through the optic cup, where it anastomoses with the annular vessel, a capillary plexus, surrounding the optic cup. The vascular network of the

hyaloid artery forms the posterior tunica vasculosa lentis, whereas the anterior counterpart is formed by branches of the annular vessel.

Infiltrating mesenchymal cells into the anterior tunica vasculosa lentis are responsible for forming the peripheral iris and the central pupillary membrane. The pupillary membrane is a solid sheet of tissue, which is continuous with the iris. This structure will form the future pupil of the iris.

During later foetal development, this vascular network is no longer required as the aqueous humour is then responsible for a nutritional supply, and therefore begins to regress. Some remnants of the pupillary membrane remain behind. These are termed persistent pupillary membranes (PPM), with the base attached to the iris collarette and the distal end free floating in the anterior chamber, contacting the corneal endothelium, lens capsule or more commonly, other areas of the iris.

PPMs are exceptionally common in neonatal foals (Barnett, 1975; Latimer *et al*., 1983; Roberts 1992), and are often seen in adult horses as an incidental finding. In a study by Plummer & Ramsey (2011) all fifty-three Miniature Horses which were examined, had persistent pupillary membranes extending from iris collarette to iris collarette.

2.2.5 Lens

Anatomy and physiology of the equine lens is similar to other mammalian species, with the three distinct areas of lens capsule, cortex and nucleus. The equine lens is large and is approximately 20x20mm with an axial length of 11-13.5mm (Whitley 2005).

The cortical region is comprised of cortical fibre cells, which are continuously being produced and moved centrally toward the nucleus. This normal ageing change of the lens is termed nuclear sclerosis and is seen as an increased opacification of the nuclear region. Nuclear sclerosis is more obvious or notable in horses 15-20 years old and older (Gilger & Hollingsworth 2016).

Opacities of the equine lens are not uncommon but if severe, can significantly decrease vision or even result in blindness. They range from small, focal embryonic remnants affecting the lens capsule to mature cataracts.

Lens opacities can be divided into two broad classifications, namely developmental or acquired opacities. Developmental or congenital opacities are related to errors which occur during embryogenesis involving either the development of the crystalline lens or the dysgenesis of the foetal vasculature. Congenital cataracts constitute the majority of these opacities.

Cataracts in any species can be congenital or acquired, with the former being considered hereditary in many breeds such as the Quarter Horse and the Morgan Horse (Beech *et al*., 1984; Beech & Irby 1985; Joyce *et al*., 1990). A study in Exmoor Ponies in Canada showed a familial trend of cataract formation, which was suggestive of a sex-linked genetic defect (Pinard & Basrur2011). In the latter, Beech *et al*. (1984) described bilateral nuclear, non-progressive cataracts, which were shown to have an autosomal dominant pattern of inheritance (Beach & Irby 1985). Spherical nuclear cataracts associated with multiple congenital abnormalities have been reported in Rocky Mountain Horses (Ramsey *et al*., 1999).

Developmental Opacities

Matthews (2000) classified developmental opacities into three broad groups on the basis of localisation, namely extralenticular, capsulolenticular and lenticular. Abnormalities were classified into one of these groups:

1) Extralenticular
- Retrolenticular fibroplasia
- Mittendorf's Dot
- PPMs involving the lens
- Pigmentation

2) Capsulolenticular
- Anterior capsular cataract
- Posterior capsular cataract
- Anterior polar cataract
- Posterior polar cataract

3) Lenticular
- Zonal cataract
- Embryonic nuclear cataract
- Foetal nuclear cataract
- Perinuclear (lamellar) cataract
- Equatorial cataract
- Sutural cataract
- Anterior cataract
- Axial cataract
- Elliptical cataract
- Complete

Acquired or Secondary Cataract

Acquired or secondary cataracts are as a result of factors external to the lens such as ocular or systemic disease, or environmental influences such as electric shock and UV radiation. They can be grouped by aetiology, such as uveitis, senility, glaucoma, neoplasia, retinal detachment and whiplash injury (Matthews 2000).

Anterior uveitis is a common cause of cataract formation and is usually associated with dense focal anterior capsular and sub capsular cataracts with or without synechiae formation (Matthews 2000). Similarly, posterior uveitis or chorioretinitis is associated with posterior capsular or sub capsular cataracts (Matthews 2000).

Senile cataracts appear to be more common in horses over the age of 18, and can affect the cortex, nucleus or appear as “Y” shaped opacities around the posterior lens sutures (Matthews 2000). Such cataracts are most often bilateral, but not always symmetrical.

Studies demonstrating the prevalence of cataracts for specific breeds include Polish Arab horses 3.3% (Paschalis-Trela *et al*., 2017), Lipizzaners 8.9% (Rushton *et al*., 2013) and racing Thoroughbreds 19.6% (Hurn & Turner 2006). Chandler *et al*. (2003) and Ireland *et al*. (2012) recorded the prevalence of cataracts as 19.3% and 58.5% respectively in geriatric horses.

2.2.6 Vitreous

Vitreal degeneration

Vitreal degeneration is also termed syneresis and appears to be more common in older horses. In a study by Chandler *et al*. (2003), 72 of 166 eyes (43.3%) in horses over the age of 15 years old had vitreal degeneration. Most cases are considered to be benign, however syneresis can result secondarily due to inflammation and result in vitreal floaters, which are considered a potential cause of head shaking behaviour (Wilkie 2011). This is yet to be proven though.

Asteroid Hyalosis

Calcium-phosphate crystal deposits can accumulate in the vitreous as asteroid hyalosis. This is an incidental, benign finding and appears to be more common in older horses.

2.2.7 Fundus

Chorioretinitis

Chorioretinitis refers to inflammation of the choroid and retina, and is the most common abnormality of the equine fundus (Wilkie 2011). These two layers, the choroid and the retina, are intimately associated and inflammation in one layer can easily lead to inflammation in the other. Lesions can be uni- or bilateral, focal or diffuse. Lesions take on a different appearance depending on chronicity, and can be focal to diffuse. Active lesions appear out of focus or hazy in appearance, white to grey in colour and are associated with oedema, cellular infiltrates, haemorrhages and even retinal detachments due to sub retinal fluid accumulation (Wilkie 2011). More chronic lesions appear as hyperreflective areas in the tapetum (Wilkie 2011). In the non-tapetum, lesions tend to be seen as depigmented areas or areas of pigment clumping (Wilkie 2011).

Typical focal chorioretinopathies are termed as ‘bullet hole’ lesions due to their appearance and can present as a single ‘bullet hole’ or multifocal lesions. These lessons are small, flat, have a dark centre and a white surrounding circular area. Histologically, retinal pigment epithelium (RPE) hyperplasia and migration of these cells into the retina is seen (Barnett 2004). It is thought that these lesions are an incidental finding, appear to be non-progressive and a definitive causative agent is yet to be associated with such findings.

Rebhun (1992) described a diffuse chorioretinitis with lesions appearing as vermiform, circular or band shaped. The classic location of diffuse chorioretinal scarring is around the peripapillary region and is termed a “butterfly’ lesion due to the classic appearance of the lesion.

Causes of chorioretinitis are numerous and can range from idiopathic to ERU to systemic disease (Wilkie 2011).

Prevalence of chorioretinal lesions varies greatly between studies. In a survey of Lipizzaner horses in Austria, 79 of 203 (38.9%) had ‘bullet hole’ lesions and only one horse had a ‘butterfly’ lesion (0.005%) (Rushton *et al*. 2013a). A study involving geriatric horses over the age of 15 years old showed a prevalence of 5 of 83 (6%) horses with peripapillary lesions and 3 of 83 (3.4%) with focal chorioretinopathies (Chandler *et al*., 2003). Australian racing Thoroughbred horses showed 108 of 204 horses had ‘bullet hole’ lesions (52.9%) and 5 of 204 had ‘butterfly’ lesions (2.5%) (Hurn and Turner 2006).

2.2.8 Optic nerve

Optic nerve head masses

Equine ocular neoplasia is uncommon, with the exception of SCC and sarcoid (Dugan 1992). Previously diagnosed tumours of the equine optic nerve head (ONH) include astrocytoma, medulloepithelioma, neuroepithelioma, and granular cell tumour (Bistner *et al*., 1983; Cutler 2000; Eagle *et al*.1978; Gelatt *et al*., 1971; Riss & Rebhun 1990; Ueda *et al*., 1993). These appear as white, grey or pink raised or pedunculated masses extending into the vitreous.

Proliferative and exudative optic neuropathy

A further differential for optic nerve head masses include proliferative and exudative optic neuropathies, as well as exudative optic neuritis. The latter is mainly described in older horses, which present with an acute onset bilateral blindness (Cutler *et al*., 2000). Proliferative and exudative optic neuropathies also occur in aged horses (>15 years), however are generally an incidental finding and rarely effect vision (Rebhun 1992). The appearance is a unilateral, white, grey or pink lesion emanating from the periphery of the optic disc. The cause is unknown, but is speculated to be a response to systemic disease (Cutler *et al*., 2000).

2.2.9 Equine recurrent uveitis

ERU is a complex, immune-mediated inflammatory condition which has the ability to result in a panuveitis (Wilkie 2011). Various reports state the prevalence of between 2-25% (Gilger & Deeg 2011), however some reports even state a prevalence as high as 56.7% (Paschalis-Trela *et al*., 2017). ERU is considered as the leading cause of blindness in horses (Gilger & Deeg 2011). The main clinical feature of ERU is multiple episodes of uveitis separated by periods of quiescence with the time period between episodes varying from months to years. Various factors play a role in the development of ERU such as inciting cause, genetics and environment. Whilst a definite aetiology is yet to be ascertained, many infectious aetiologies including bacterial, viral, parasitic and protozoal have been suggested with Leptospirosis being one of the most investigated causes (Brandes *et al*., 2007; Brem *et al*.,1999; Faber *et al*., 2000; Pearce *et al*., 2007; Verma *et al*., 2005; Wollanke *et al*., 2001).

The clinical signs associated with ERU are numerous and can involve both the anterior and/or the posterior segment of the globe. Acute episodes of ERU are characterised by the typical clinical signs of uveitis (Gilger & Deeg 2011). Following repeated bouts of ERU, residual clinical signs such as keratin precipitates, fibrosis, synechiae formation, lens capsule pigmentary deposits and cataract formation become evident (Gilger & Deeg 2011). Additionally, glaucoma is commonly diagnosed in ERU cases (Gilger & Deeg 2011). Insults to the posterior segment are more

noticeable during episodes of quiescence, as the ocular media are opaque during active episodes. Such signs include vitreal degeneration, chorioretinal scarring, retinal degeneration or detachment (Gilger & Deeg 2011). Typical chorioretinal scarring is seen either as small, multifocal circular areas of depigmentation with a hyper pigmented centre which take on a ‘bullet hole’ appearance, or as larger peripapillary areas of hypopigmentation ('butterfly' lesions) (Gilger & Deeg 2011).

Whilst ERU can manifest in any breed of horse, Warmblood horses may be genetically predisposed. Deeg *et al*. (2004) showed that German Warmbloods carrying the major histocompatibility complex (MHC) class I ELA-A9 haplotype were more susceptible to develop ERU than horses of the same breed without this haplotype. Forty-one per cent of the ERU-affected group were positive for ELA-A9 haplotype. Kulbrook *et al*. (2013a), using a genome wide association study (GWAS) in 114 German Warmblood horses, identified loci which may confer a risk of developing ERU. A significant single nucleotide polymorphism (SNP) on horse chromosome 20 at 49.3 Mb, with IL-17A and IL-17F being the closest genes, which may have a role in the pathogenesis of ERU. A further SNP was identified on horse chromosome 18 nearby to the crystalline gene cluster CRYGA-CRYGF which may be indicative for cataract formation in ERU cases.

2.3 Outcome of the literature review

This review demonstrates that ocular abnormalities in various populations of horses are ubiquitous and the need for more routine ophthalmic examinations is obvious. Certain conditions may be irreversible at time of diagnosis, may respond to treatment or may explain an unfavourable trait or habit. Current studies are limited by subject number and limited breeds. Future studies are needed which involve larger cohorts. Here we shall assess and describe the prevalence of ophthalmic pathology in the Warmblood breed for the first time.

2.4 Project Justification/Benefits arising from the project

Information gained from this project can be used to determine the prevalence of ophthalmic disease in Warmblood horses in South Africa, and to promote earlier diagnosis and treatment.

Chapter 3

AIMS, OBJECTIVES, HYPOTHESES, AND BENEFITS

3.1 Aims and Objectives

- To record and document the prevalence of ophthalmic abnormalities in populations of Warmblood horses living in South Africa.
- To speculate if abnormalities may have a congenital/hereditary component.
- To determine if chorioretinal and lens abnormalities are age-related.
- Establish normal IOP and STT values in clinically normal Warmblood horses.
- To document normal anatomic variations.
- Data to be used as a part of the MMedVet (ophthalmology) research components for Dr. Ramona Allen

3.2 Hypotheses

- Primary hypothesis
- Ocular abnormalities occur in Warmblood horses in South Africa.
- The percentage of ocular abnormalities detected is age-related.

- Null hypothesis
- Ocular abnormalities do not occur in Warmblood horses in South Africa.
- The percentage of ocular abnormalities detected is not age-related.

Chapter 4

MATERIALS AND METHODS

4.1 Materials and Methods

4.1.1 Population

Populations of Warmblood horses at various studs, liveries and riding schools in the Gauteng and North West provinces of South Africa were examined. Horses were grouped in age categories 0-6, 7-9, 10-15, 16-30 years. Horses were selected by the manager or owner of the establishment for examination as not all of the horses at the various establishments were Warmbloods. Individuals with a history of ocular pathology were excluded.

All yards were located in the suburb of Kyalami, Gauteng except for one yard which was located in the Skeerpoort area of the North West Province.

4.1.2 Study design

A descriptive, observational study design.

A complete ocular examination of both eyes was performed without sedation or regional anaesthesia. Examination took place in a darkened stable with and without pupil dilation. The following were included in the examination:

1) Tear production was measured using a STT (Intervet, Inc., Merck Animal Health).

2) Tonometry was performed using a TonoVet tonometer (Icare). This measurement was taken prior to pupil dilation with Tropicamide (Mydriacyl; Alcon).

3) Examination of the anterior chamber of the eye.

4) A single drop of 1% Tropicamide (Mydriacyl; Alcon) was applied to each cornea followed by a 30 minute waiting period.

5) Ophthalmic examination took place using slit lamp biomicroscopy (Keeler PSL Classic Portable Slit Lamp (Keeler, U.K.) to assess the anterior and posterior segments of the globe. The light

shape settings for the Keeler are 0.15, 0.5, 0.8 and 1.6 slits; a round beam of 12mm and a 1mm square beam.

6) Examination concluded with 2% fluorescein sodium (Minims; Bausch and Lomb) being applied to the eye to check for possible corneal pathology. The cornea and conjunctiva was examined with a cobalt blue light source.

4.1.3 Study procedure

A darkened stable setting was used to facilitate all examinations. A general “hands off” examination was first performed to assess globe size and position, symmetry between the two eyes and eyelids. Following this dPLR, cPLR, the menace response and dazzle reflex was tested. The rest of the examination included a Schirmer tear test, measurement of IOP, tropicamide application, slit lamp biomicroscopy, direct ophthalmoscopy, and concluded with a fluorescein dye test.

4.1.4 Staff and facilities

This research project involved:

• Dr Ramona Allen
- Ophthalmic examinations, data collection and documentation

• Dr Antony Goodhead
- Supervisor; ophthalmic examinations

• Prof Peter Thompson
- Statistical analyses and data evaluation

• Stable yard managers at the various locations

• Stable hands at the various locations
- Assistance with handling of the horses

4.2 Data analysis

Data collected was recorded on data collection sheets (Appendix 1) and captured using Apple Numbers version 6.2.

Age was categorised into 4 quartiles (0-6 year old; 7-9 year old; 10-15 year old; 15+ years old). Prevalence of each lesion was calculated at the eye level and at the horse level, with 95% confidence limits.

Prevalence of cataracts and chorioretinal lesions was compared between age categories using logistic regression with robust standard errors to accommodate for clustering of eyes within horses. Similarly, count of focal chorioretinopathy lesions were compared between age categories using Poisson regression with robust standard errors. IOP was log-transformed to achieve normality. Both IOP and STT were compared between left and right eyes and between age categories using linear mixed models with animal and stable as nested random effects. Statistical analysis was done using Stata 15.1 (StataCorp, College Station, TX, U.S.A.). Significance was assessed at $P < 0.05$.

4.3 Ethical considerations

Application to the Animal Ethics Committee of the University of Pretoria was made for approval of this study. Written consent for ophthalmic examination was obtained from all horse owners or stable yard managers (Appendix 2).

Chapter 5

RESULTS

5.1 Results

A total number of 104 horses were examined. Tables 1 and 2 indicate the age and gender distributions.

Table 1. Gender distribution of horses examined

Group	Mare	Gelding	Stallion
Total	33	63	8

Table 2. Age and gender distribution of horses examined

Gender	0.4-6yr	7-9yr	10-15yr	15+yr	Total
Mare	7	11	8	7	33
Gelding	16	17	15	15	63
Stallion	3	2	2	1	8
TOTAL	26	30	25	23	104

The mean STT and IOP values are contained in Table 3. A mean of 25.7mm/min ± 6mm/min was recorded with the lowest value recorded being 8mm/min and the highest being 35mm/min, the maximum recordable value. The mean IOP recorded was 34.4mmHg ± 10.8mmHg with the lowest and highest values being, 15mmHg and 70mmHg respectively.

Table 3. Mean STT and IOP values of total eyes of horses examined

Variable	Mean	SD	Minimun	Maximum
STT (mm/min)	25.7	6	8	35
IOP (mmHg)	34.4	10.8	15	70

STT = Schirmer tear test IOP = Intraocular pressure

The results for the mean STT and IOP values between right and left eyes are contained in Table 4. No statistical difference ($P > 0.05$) was noted between right and left eyes for either variable.

Table 4. Mean STT and IOP values of the right and left eyes of horses examined

Variable	Right eye	Left eye	*P* - value
STT (mm/min)	24.4	25	0.6
IOP (mmHg)	36.5	32.7	0.2

STT = Schirmer tear test IOP = Intraocular pressure

The results for the mean STT and IOP values between different genders are contained in Table 5. No statistical difference ($P > 0.05$) was noted between genders for either variable.

Table 5. Mean STT and IOP values between gender examined

Variable	Mare	Gelding	Stallion
STT (mm/min)	25.5	24.5	22.3
IOP (mmHg)	32.7	35.5	32.8

STT = Schirmer tear test IOP = Intraocular pressure

The results for the mean STT and IOP values between age groups is contained in Table 6. No statistical difference ($P > 0.05$) was noted between age groups for for either variable.

Table 6. Average ocular values between age groups examined

Variable	0.4-6yr	7-9yr	10-15yr	15+yr
STT (mm/min)	24.6	24.6	25.1	24.4
IOP (mmHg)	36.9	34.3	33.1	33.3

STT = Schirmer tear test IOP = Intraocular pressure

The prevalence of the described ocular lesions, at both the eye and horse level, are contained in Table 7. Most lesions were unilateral, however lesions which were recorded bilaterally included conjunctivitis in 5 horses, iris to iris persistent pupillary membranes in 84 horses, iris hyperpigmentation 6 horses, iris cyst in 6 horses, cataracts in 5 horses, chorioretinal lesions in 35 horses, vitreal degeneration in 2 horses, nuclear sclerosis in 7 horses and asteroid hyalosis in 2 horses.

Table 7. Prevalence and confidence intervals of ocular lesions at the eye and horse levels in 104 Warmblood horses (208 eyes)

	AT EYE LEVEL			AT HORSE LEVEL		
Variable	**N**	**Prevalence**	**95% confidence interval**	**N**	**Prevalence**	**95% confidence interval**
Corneal ulceration	0	0	0-0.2	0	0	0-0.04
Eyelid scar	2	1%	0.001-0.03	2	1.9%	0.002-0.07
Chalazion	1	0.5%	0.0001-0.3	1	1%	0.0002-0.05
Eyelid mass	2	1%	0.001-0.03	2	1.9%	0.002-0.07
Conjunctivitis	12	5.8%	0.03-1	7	6.7%	0.03-0.1
Conjunctival follicles	2	1%	0.01-0.4	1	1%	0.0002-0.1
Conjunctival mass	1	0.5%	0.0001-0.3	1	1%	0.0002-0.1
Corneal fibrosis	2	1%	0.001-0.03	2	1.9%	0.002-0.07
Linear keratopathy	1	0.5%	0.0001-0.03	1	1%	0.0002-0.1
Persistent pupillary membranes	139	66.7%	0.6-0.7	75	72.1%	0.6-0.8
Corpora nigra hyperplasia	1	0.5%	0.0001-0.03	1	1%	0.0002-0.1

	AT EYE LEVEL			AT HORSE LEVEL		
Variable	**N**	**Prevalence**	**95% confidence interval**	**N**	**Prevalence**	**95% confidence interval**
Torn corpora nigra	1	0.5%	0.0001-0.03	1	1%	0.0002-0.1
Iris hyperpigmentation	16	7.7%	0.04-0.1	10	9.6%	0.05-0.2
Posterior synechiae	1	0.5%	0.0001-0.3	1	1%	0.0002-0.1
Uveal cyst	3	1.4%	0.002-0.4	2	1.9%	0.002-0.07
Lens capsule opacity (embryological remnant)	4	1.9%	0.01-0.1	4	3.9%	0.01-0.1
Axial cataract	6	2.9%	0.01-0.1	4	3.9%	0.01-0.1
Anterior capsular cataract	7	3.8%	0.02-0.1	6	5.8%	0.02-0.1
Posterior capsular cataract	1	0.5%	0.0001-0.03	1	1%	0.0002-0.1
Anterior polar cataract	3	1.4%	0.003-0.04	3	2.9%	0.006-0.1
Posterior polar cataract	3	1.4%	0.003-0.04	3	2.9%	0.006-0.1
Posterior cortical cataract	1	0.5%	0.0001-0.03	1	1%	0.002-0.1
Perinuclear (lamellar) cataract	1	0.5%	0.0001-0.03	1	1%	0.0002-0.1
Nuclear sclerosis	14	6.7%	0.04-0.1	7	6.7%	0.03-0.1
Total Cataract	19	9.1%	0.1-0.13	13	12.5%	0.1-0.2
Vitreal degeneration	8	3.9%	0.02-0.1	6	5.8%	0.02-0.1
Vitreal herniation	1	0.5%	0.0001-0.03	1	1%	0.0002-0.1
Asteroid hyalosis	4	1.9%	0.01-0.1	2	1.9%	0.002-0.07
Non-peripapillary chorioretinopathy	14	6.7%	0.04-0.1	12	11.5%	0.06-0.2
Peripapillary chorioretinopathy (‘butterfly’ lesions)	7	3.4%	0.01-0.07	6	5.8%	0.02-0.1
Focal chorioretinopathy (‘Bullet hole’ lesions)	88	42.3%	0.4-0.45	56	53.9%	0.4-0.6
Total chorioretinal lesions	100	48.1%	0.4-0.6	65	62.5%	0.5-0.7

	AT EYE LEVEL			AT HORSE LEVEL		
Variable	**N**	**Prevalence**	**95% confidence interval**	**N**	**Prevalence**	**95% confidence interval**
Linear chorioretinal scar	3	1.4%	0.003-0.04	3	2.9%	0.01-0.08
Optic nerve head mass	1	0.5%	0.0001-0.03	1	1%	0.002-0.05s

Table 8 indicates the prevalence of cataract for the various ages groups, which appeared to increase with age, being significantly higher in the 10-15yr and 15+yr age categories when compared to the 7-9yr category. Statistical comparison of total cataract lesions between age categories are versus a baseline group. This was done using logistic regression with robust standard errors to account for clustering of eyes within animals.

Table 8. Total cataracts by age group at the eye level

Age category	**N**	**Prevalence**	***P*-value**
0.4-6yr	2	3.9%	0.550
7-9yr*	1	1.7%	-
10-15yr	7	14%	0.039
15+yr	9	19.6%	0.018

*baseline group

Table 9 indicates the prevalence of chorioretinopathy lesions for the various age groups, which appeared to increase with age, being statistically significant in the 7-9yr and 15+yr age categories when compared to the 0.4-6yr category. Statistical comparison of total chorioretinopathy lesions between age categories are versus a baseline group. This was done using logistic regression with robust standard errors to account for clustering of eyes within animals.

Table 9. Total chorioretinopathy lesions by age group at the eye level

Age category	N	Prevalence	*P*-value
0.4-6yr*	16	31.8%	-
7-9yr	32	53.3%	0.050
10-15yr	21	42%	0.316
15+yr	31	67.4%	0.003

*baseline group

Table 10 indicates the prevalence of non-peripapillary chorioretinopathy lesions for the various age groups. Statistical comparison of non-peripapillary chorioretinopathy between age groups are versus a baseline group. No significant difference ($P > 0.05$) was noted in any of the age categories when compared with the 7-9yr category. This was done using logistic regression with robust standard errors to account for clustering of eyes within animals.

Table 10. Non-Peripapillary chorioretinopathy numbers by age group at the eye level

Age category	Prevalence	*P*-value
0.4-6yr	57.7%	0.875
7-9yr*	5%	-
10-15yr†	-	-
15+yr	17.4%	0.051

* Baseline group

† No lesions recorded for this age group

Table 11 indicates the prevalence of peripapillary chorioretinopathy lesions for the various age groups. Prevalence of peripapillary chorioretinopathy lesions appeared to increase with age and was highest in the 15+yr age category, however no significant difference ($P > 0.05$) was noted in any of the age categories when compared with the 7-9yr category. Statistical comparison of peripapillary chorioretinopathy between age groups are versus a baseline group. This was done

using logistic regression with robust standard errors to account for clustering of eyes within animals.

Table 11. Peripapillary chorioretinopathy (‘butterfly’ lesion) numbers by age group at the eye level

Age category	Prevalence	*P*-value
0.4-6yr	3.9%	0.9
7-9yr*	3.3%	-
10-15yr †	-	-
15+yr	6.5%	0.5

* Baseline group

† No lesions recorded for this age group

Table 12 indicates the prevalence of focal chorioretinopathy lesions for the various age groups, which appeared to increase with age, being statistically significant in the 15+yr age category when compared to the 0.4-6yr category. Statistical comparison of focal chorioretinopathy lesions between age categories are versus a baseline group. This was done using logistic regression with robust standard errors to account for clustering of eyes within animals.

Table 12. Focal chorioretinopathy (‘bullet hole’) lesions by age group at the eye level

Age category	Prevalence	*P*-value
0.4-6yr*	23.1%	-
7-9yr	45%	0.056
10-15yr	42%	0.093
15+yr	60.9%	0.004

* Baseline group

Statistical comparison of focal chorioretinopathy lesions between age categories uses a *p*-value versus a baseline group. This is done using a Poisson regression model with robust standard errors to account for clustering of eyes within animals.

The number of 'bullet hole' lesions was shown to increase with age, with counts being significantly higher in 7-9yr and 15+yr compared to the youngest age category (Table 13. and Fig. 6).

Table 13. Comparison of counts of focal chorioretinopathy ('bullet hole') lesions by age group at the eye level

Age category	*P*-value	Min	Max	Median	Interquartile range
0.4-6yr*	-	0	18	0	0
7-9yr	0.037	0	20	0	0-4
10-15yr	0.108	0	20	0	0-3
15+yr	0.001	0	16	5	0-7

* Baseline group

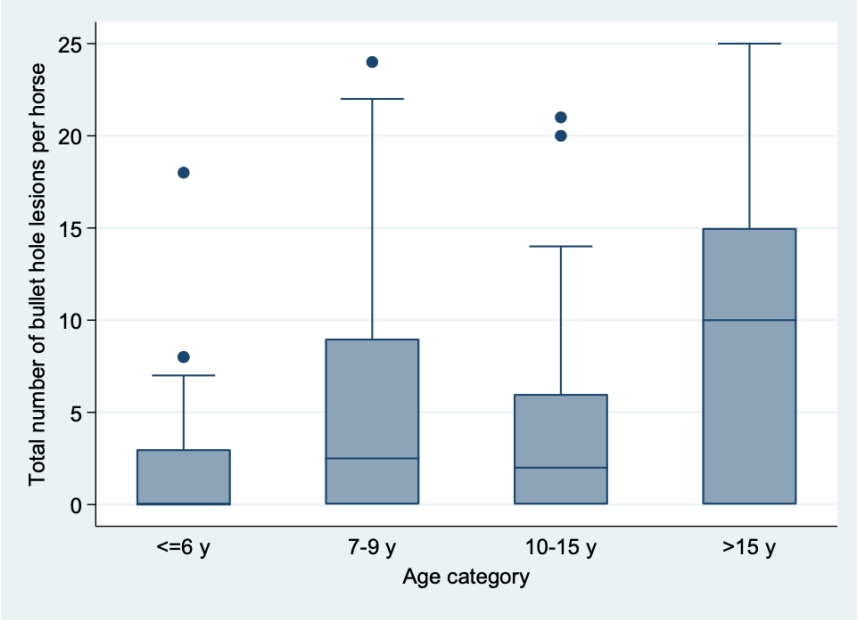

Fig. 6. Box plot of Total number of focal chorioretinopathy ('bullet hole') lesions per horse for different age categories.

All horses in this study were visual bilaterally, and all had normal dPLR AND cPLR, with the exception of one eye (Fig. 9.), which had multiple anterior segment defects due to a presumed previous inflammatory episode. The menace response was positive in all eyes examined. Figures 7 and 13 show a normal anterior segment of a horse's globe and fundus, respectively. Figures 8, 9, 10, 11, 12, 14, 15, 16, 17 and 18 illustrate some of the conditions observed in this study.

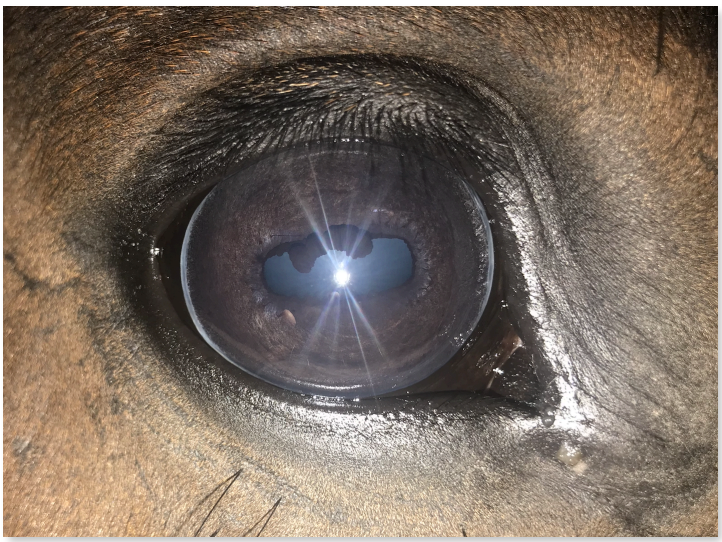

Fig. 7. Normal anterior segment of a horse

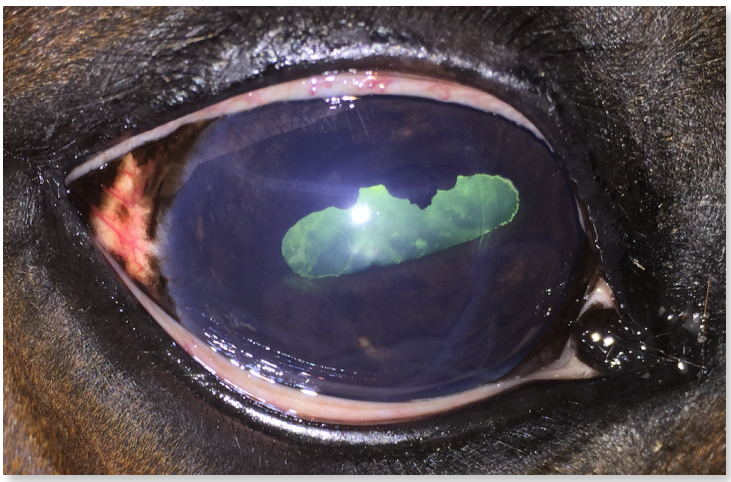

Fig. 8. Linear Keratopathy

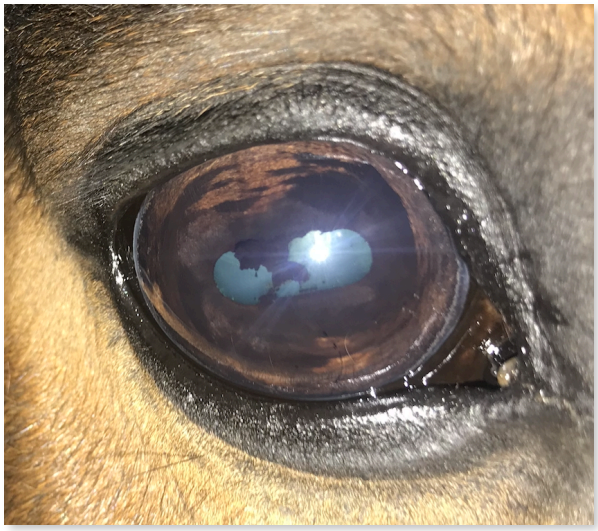

Fig. 9. Multiple anterior segment defects and cataract formation

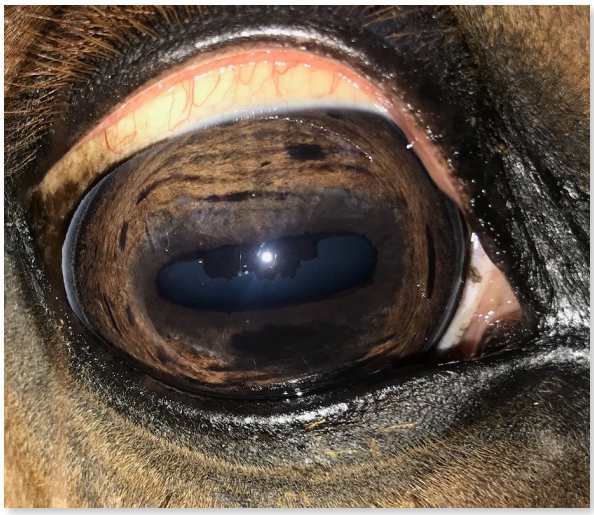

Fig. 10. Hyperpigmentation of the iris

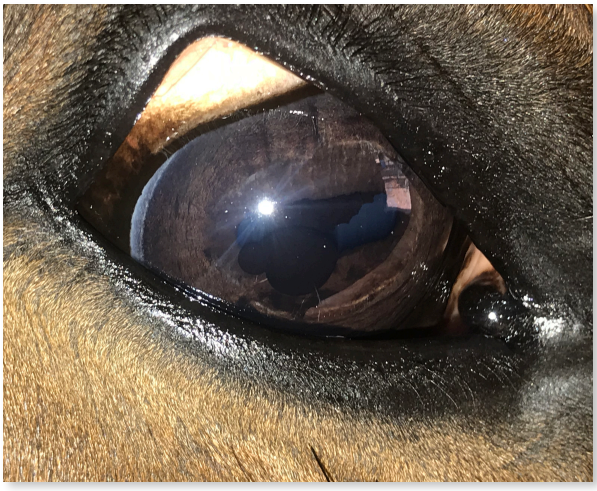

Fig. 11. A uveal cyst

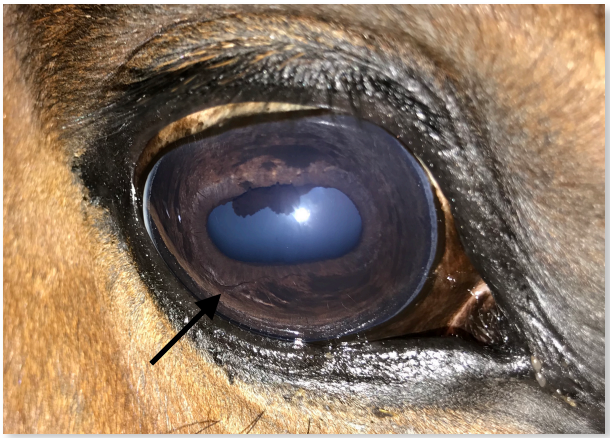

Fig. 12. Iris to iris persistent pupillary membrane (arrow)

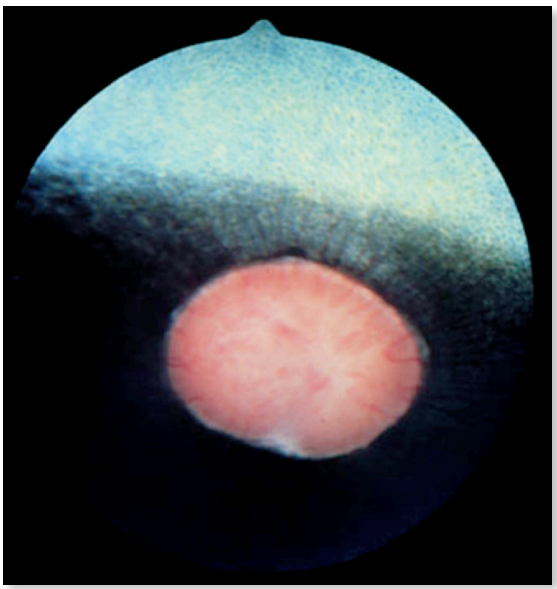

Fig. 13. Normal fundus of a horse (Wilkie 2011)

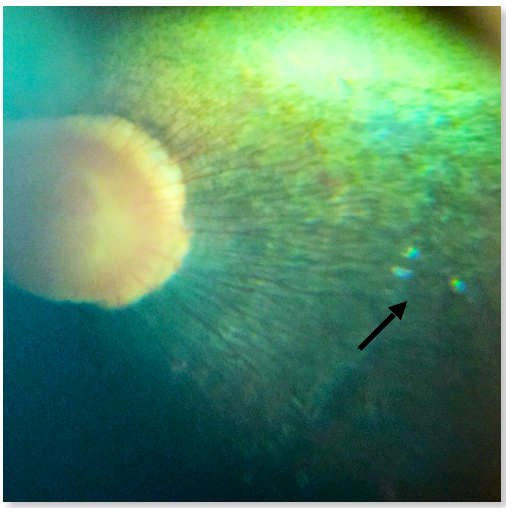

Fig. 14. Focal ‘bullet hole’ chorioretinopathy (arrow)

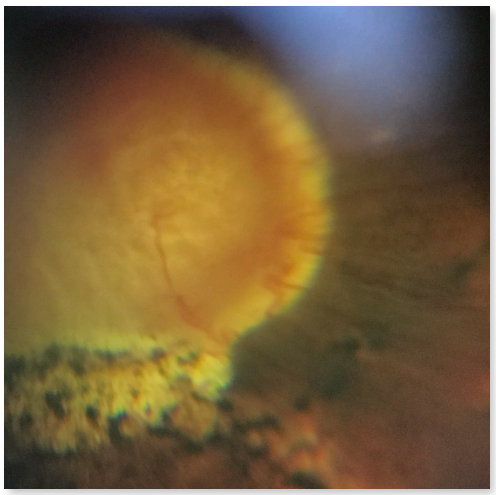

Fig. 15. Peripapillary 'butterfly' chorioretinopathy

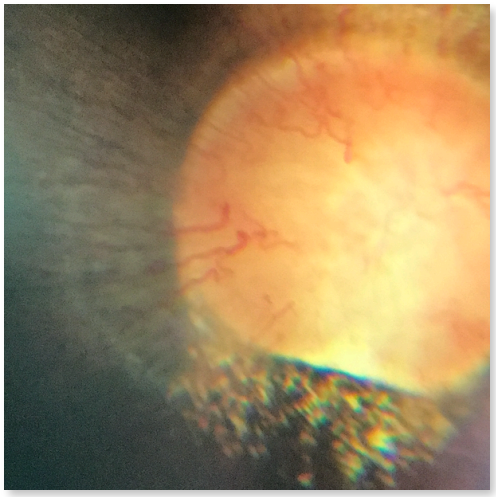

Fig. 16. Peripapillary 'butterfly' chorioretinopathy

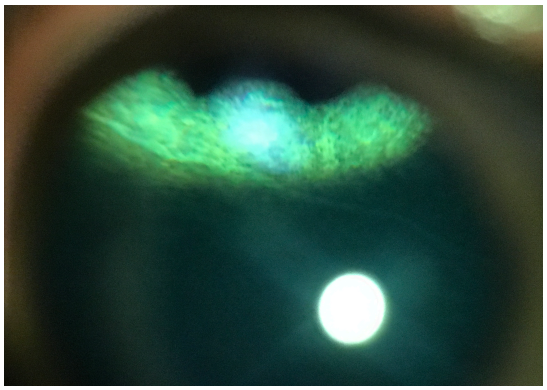

Fig. 17. Linear chorioretinopathy

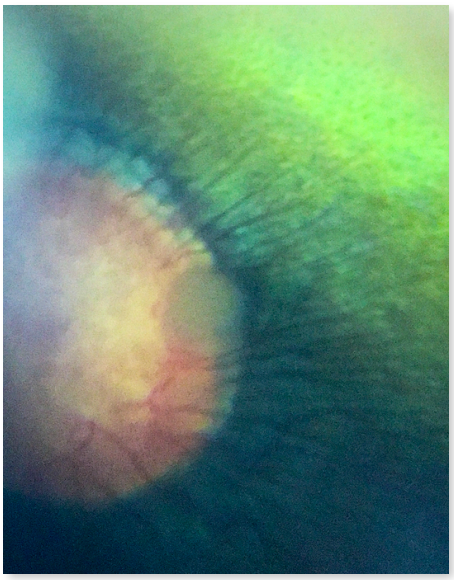

Fig. 18. Optic nerve head mass

Chapter 6

DISCUSSION

6.1 Discussion

6.1.1 STT

Schirmer tear tests are not always routinely performed during ophthalmic examinations in horses, as KCS is rare in this species, however is most commonly reported with trauma to cranial nerves V or VII (Gilger & Stoppini 2011). The large variation in STT values in this study demonstrates the importance of performing this test and that cases of KCS may go undiagnosed. Measurements which may be borderline, in the range 10-15mm/min, perhaps should be repeated and confirmed. Individuals with results below 10mm/min, together with clinical signs, should be investigated further for underlying causes of KCS and a treatment plan formulated.

Our data was in line with data recorded by Beech *et al*. (2003) who also found no difference between left and right eyes, and with age having no significant effect. This is however in contrast to findings by Piccione *et al*. (2003), who found a significant difference left and right eyes, as well as between genders.

6.1.2 Tonometry

All horses were examined conscious and no sedation was utilised in this study, therefore IOP should be interpreted with caution. IOP is a valuable tool in diagnosing conditions such as glaucoma and uveitis.

The values reported in this study are significantly higher than previously recorded by Knollinger *et al*. (2005), who recorded a mean of 22.1 ± 5.9 mm Hg (range, 10 to 34 mm Hg) in normal, conscious and unsedated horses using rebound tonometry with a Tono-Vet apparatus. It has however been suggested that three separate readings should be taken and the average recorded (Gelatt 2007). These should have less than 5% standard error. Horses without an auriculopalpebral block may have elevated IOP due to eyelid tension, however a study by Van der Woerdt *et al.* (1995) showed no significant difference in IOP before and after auriculopalpebral blocks. Other factors such as circadian rhythm influencing IOP may have resulted in the broad range noted in this study as horses examined where not always examined at a similar time of the day. Bertolucci *et al.* (2009) demonstrated the effect of circadian rhythm on IOP with higher readings being

recorded during the light phase and the highest readings being recorded at the end of the light phase.

6.1.3 Conjunctiva

Conjunctival lesions were reported in 7.2% of eyes with the majority of lesions being conjunctivitis. Conjunctival follicles were seen bilaterally in a single horse, which may be indicative of an immune mediated aetiology. Conjunctivitis was noted in 7 horses and was more often bilateral. Causes of conjunctivitis are classified as primary or secondary, with the former resulting from various causes such as infectious, immune-mediated, trauma and solar induced (Gilger & Stoppini 2005). If one had to speculate with regards to a cause of the conjunctivitis noted in this study, an allergic aetiology would be presumed,.The Kyalami area is known to be exceptionally dry and dusty at particular times of the year supporting the presumed allergic aetiology.

6.1.4 Cornea

All horses underwent a fluorescein dye test to assess corneal integrity. No corneal ulceration was detected throughout this study. Two eyes had corneal fibrosis and one eye had a linear keratopathy, making the overall prevalence of corneal pathology very low at 1.4%. This is in contrast to a study by Rushton *et al*. (2013a) which noted a high percentage (21.7%) of corneal opacities in Lipizzaner horses, whereas Hurn & Turner (2006) demonstrated a low incidence of corneal pathology in Thoroughbred horses. Rushton *et al*. (2013a) speculated an underlying immune-mediated process in the Lipizzaner breed.

6.1.5 Lens

Nuclear sclerosis was reported bilaterally in 7 horses. There are few reports of nuclear sclerosis in horses, however Plummer & Ramsey (2011) only reported nuclear sclerosis in Miniature Horses over the age of 13 years, whereas Chandler *et al.* (2003) reported a prevalence of 7.2% in horses and ponies over the age of 15 years. Andrysikova *et al*. (2019) reported a high prevalence, 18.4%, in Old Krabruber horses, however there was no indication of age of these horses. Other studies indicate a lower prevalence (Rushton *et al*., 2013a; Pinard & Basrur 2011). Some studies may not have reported on age-related variations and therefore no data is available.

The prevalence of cataracts in this study was 9.1% at the eye level and 12.5% at the horse level, neither of which were statistically significant. This is higher than reported for Lippizaner horses with a prevalence of 8.5% at the horse level (Rushton *et al*., 2013a). Interestingly, Hurn & Turner (2006) reported a prevalence of 19.6% at the horse level in racing Thoroughbreds. This is unusual as this

horse population is much younger than the population reported in this study. Thoroughbreds in training may be more prone to trauma due to the nature of their utility which may explain the difference in age. The most common cataract noted in this study was an anterior capsular cataract followed by an axial cataract, both of which are thought to be non-progressive (Matthews 2000). However, the only way to monitor progression would be sequential examinations. Five out of 13 horses affected by cataracts were affected bilaterally (38.5%). This may suggest a possible congenital or hereditary link, but further research would be needed to ascertain this.

The prevalence of cataracts increased with age, however this was only statistically significant in the 10-15yr and 15+yr age categories. This would suggest that the cataract formation in Warmblood horses is indeed age related, and less likely to have a congenital or hereditary component.

6.1.6 Fundus

All chorioretinal lesions noted were chronic. The prevalence of chorioretinal lesions was documented in 48.1% of eyes and 62.5% of horses. This is similar to a study involving Thoroughbred horses which documented 63.2% of horses having chorioretinal lesions (Hurn & Turner 2006). However, it is more than that reported in Lippizaner horses, which had a prevalence of 37.5% (Rushton *et al*., 2013a). Geriatric horses and ponies over the age of 15 years had a 49.4% prevalence of retinal lesions (Chandler *et al*., 2003). A subsequent study in geriatric horses showed senile retinopathy in 33.7% of horses examined (Ireland *et al*., 2012).

Chorioretinal lesions were divided into non-peripapillary, peripapillary and focal chorioretinopathies. Non-peripapillary chorioretinal lesions were documented in 6.7% of eyes and 11.5% of horses. Peripapillary (‘butterfly' lesions) chorioretinopathy lesions were seen in 3.4% of eyes and in 5.8% of horses. This is higher than reported for the Lippizaner (0.5% at the horse level) and Thoroughbred breeds (2.4% at the horse level) (Hurn & Turner 2006; Rushton *et al*., 2013a). The incidence of peripapillary chorioretinopathy was highest in the 15+yr age group, however not statistically significant. Chandler *et al*. (2003) reported peripapillary lesions in 6% of eyes in geriatric horses which is similar to the results of this study.

‘Butterfly’ lesions are usually seen unilaterally and have been speculated to result from ERU, however this is yet to be proven. These lesions have a more significant effect on vision due to their proximity to the ONH and a significant number of RGC axons (Cutler *et al*., 2000).

Focal (‘bullet hole’) chorioretinopathies lesions were reported in the majority of horses with a prevalence of 53.9%. This is significantly higher than reported for Lippizaners, which was 29.6%, but similar to that reported in racing Thoroughbreds (52.9%) (Hurn & Turner 2006; Rushton *et al*., 2013a). The highest prevalence in this study was reported in the 15yr+ group. In addition to this, it

was shown the number of ‘bullet holes’ noted indeed increased with age. The exact cause of ‘bullet hole’ lesions is speculated with aetiologies varying from ischaemia, inflammation and infarction (Matthews 2004). Experimental infection with EHV-1 in foals was shown to induce similar lesions (Slater *et al*., 1992). The non-tapetal, circular lesions in the ventral fundus represents loss of normal retinal architecture with a central area of RPE hyperplasia. Their effect on vision has drawn much attention and speculation. Allbaugh *et al*. (2014), however showed that these lesions do not appear to affect retinal function. Mathes *et al.* (2012) reported that horses with depigmented punctate chorioretinal foci, in the absence of other fundic pathology, did not have an increased likelihood of intraocular disease or ERU when compared to horses with normal ocular fundi.

6.1.6 Miscellaneous

Other lesions documented had a low prevalence, with the exception of iris pigmentation and iris to iris PPMs, which are remnants of the normal embryonic vascular structures arising from the iris collarette, but fail to regress. These were reported in the majority of horses examined in this study, however they are not documented in prevalence studies as they represent a normal anatomical variation. Uveal cysts were reported in 2 horses, with one horse being affected bilaterally. These are often small and infrequently affect vision. Large cysts may block the pupil and visual axis, and Gilger *et al*. (1997) reported resolution of visual impairment or noted behavioural changes after removal of uveal cysts using a neodymium:yttrium-aluminum-garnet (Nd:YAG) laser.

6.2 Study Limitations

This study involved 104 horses, however further studies could potentially include much larger numbers of Warmblood horses. The vast majority of horses examined were from a concentrated area and therefore may be more prone to conditions with an environmental influence. Additionally, 11 of the horses examined were from a stud farm and may have been related, and if so, there may be a genetic influence on the lesions seen.

There was a significant difference in the distribution of lesions based on gender, as most horses in commercial liveries and riding schools are geldings, due to their preferred temperament. This may result in an inaccurate representation of lesions which may have a gender basis, such as ocular trauma resulting from lively behaviour. Futures studies should eliminate this bias by choosing a population with equal numbers in each gender category.

Horses were grouped into age category quartiles in order to incorporate equal numbers of horses into each of the four groups. This resulted in an unequal age distribution between groups. The addition of younger foals would provide a better age distribution, and age groups should be of equal proportions.

Both IOP and STT were measured at various times of the day and year, which may have affected readings, as circadian rhythm has been shown to have an affect on IOP (Bertolucci *et al*., 2009). Seasonal differences in IOP and STT have not been proven, however further studies may be needed.

With regards to focal chorioretinal (‘bullet hole’) lesions, some individuals had large numbers of lesions, and therefore may have been counted incorrectly leading to an underestimation.

Chapter 7

CONCLUSION

7.1 Conclusion

The Warmblood horse is a popular breed all over the world and can be trained to be used in a multitude of equestrian sporting events. This survey of ocular pathology in Warmblood horses serves as an assessment within a breed not previously investigated. The findings in this study indicate that Warmblood horses in South Africa demonstrate ocular pathology, with chorioretinal lesions being significantly overrepresented. Such lesions have the ability to result in visual disturbances and even blindness if extensive. This finding emphasises the importance of ocular examinations, particularly as a part of a prepurchase examination or when a history of behavioural changes has been noted. Cataracts observed varied in type and were shown to increase in prevalence with age. Additionally, a normal IOP and STT values was been established in the Warmblood breed.

Future research into the pathophysiology and pathoaetiology of prevalent lesions, such as chorioretinopathies and cataract formation, is indicated. This may provide us with information, or even a link with conditions such as ERU. Many conditions are thought to possess a hereditary component and this forms a basis for future genetic studies.

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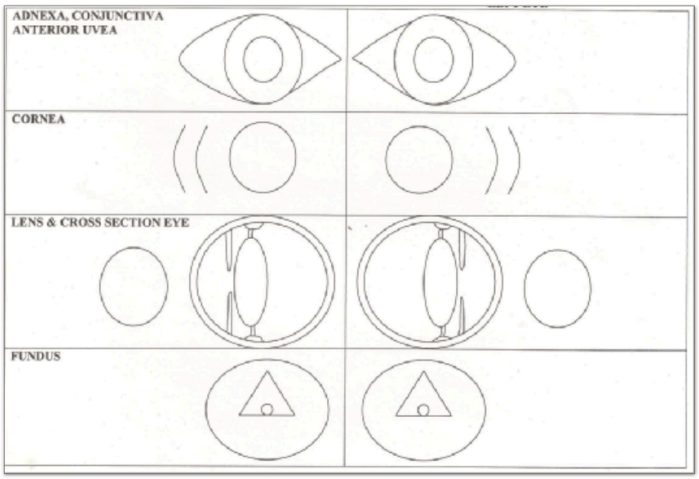
ADDENDUMS

Appendix 1: Data collection sheet

CASE NO.:		ANIMAL	
Initials of examiner:		Name of horse:	
Date and time of examination:		Breed:	..... Warmblood
Microchip #:		Sire breed (if known):	
Name of owner:		Dam breed (if known):	
Contact number:		Sex:	
Name of stable manager:		Date of birth/Age:	
Contact number:		Colour:	
Yard/stable name:		Markings:	
Stable/Box No.:		Horse utility:	
Housing Type:		Pedigree certificate (if available):	
	EXAMINATION FINDINGS		
	OD	OS	Comment
DPR			
CPR			
MENACE REFLEX			
DAZZLE REFLEX			
STT			
TONOMETRY			
FLUORESCEIN			
DIAGNOSTIC FINDINGS			
FURTHER COMMENTS			
If potential vision threatening abnormality detected, ask about behaviour.			



Appendix 2: Client consent form

Client Consent Form - Research Study

Study Title:
Incidence of ocular pathology in warmblood horses (*Equus caballus*) in South Africa.

Primary investigator:
Dr. Ramona Allen
082 6622 462
ramona@animaleyehospital.co.za

The Faculty of Veterinary Science at the University of Pretoria is considered a centre for clinical excellence and prides itself in the various innovative areas of research. Here you are invited to participate in such research by enrolling your animal a research study. Participation is on a voluntary basis and is not compulsory. Should you wish to withdraw your animal at any time there will be no penalties.

Study aim:
Our aim is to understand more about warmblood eyes, what ocular conditions they more routinely are afflicted by and whether or not such conditions may affect there vision and performance.

Study Design:
A full ophthalmic examination will be performed on your horse. We will need to dilate both pupils for this to occur by instilling one drop of medication (Tropicamide, Mydriacyl, Alcon) into both eyes. No sedation will be necessary. An examination will take between 5-15 minutes.

Study benefits:
The incidence of various ocular anomalies can be estimated in warmblood horses and how this may their influence on vision.

Study risk:
There are no direct risks to your animal, however the pupils may remain dilated for up to 12 hours therefore we advise that horses do not compete in the 12 hours after examination. The eyedrops used in this study do not generally cause any discomfort to the animal.

Confidentiality:
Data collected in this study is confidential and will only be made available to the owner of the animal at request. Should this data be published or presented, all information shall remain confidential and will not be discussed in any such way as to identify a specific animal. Upon signing this form, you give your consent and acknowledge the nature of this research and permit this animal to be included in this study.

The above information has been read and understood, and I give consent for _____

_____ (animal's name and/or microchip number) to participate in this study.

Owner of animal or Stable Manager:

Name: _____

Signature: _____Date: _____

Did you receive a copy of the consent form? ☐

Primary Investigator Signature:

Name: DR RAMONA ALLEN

Signature _____ Date: _____

Witness 1:
Name: _____

Signature _____ Date: _____

Witness 2:
Name:_____

Signature _____ Date: _____

This research project has been reviewed and approved by the Animal Ethics Committee, Faculty of Veterinary Science, University of Pretoria.

Should you have any queries or concerns with regards to the study, whilst do not hesitate to myself on the number below.

Dr. Ramona Allen
BSc (Med) Hons BVSc MMedVet (Ophthalmology resident)
Faculty of Veterinary Science
University of Pretoria
Cell: 082 6622 462

You will be provided with a copy of this form to keep for your records.

Please tick either/both the following boxes should you wish to:

☐ **Would you like to receive a report of any vision threatening abnormal ocular findings of your animal?**

☐ **Would you like to receive the final results of this study?**

Please provide us with a contact number and email should either of the above boxes be ticked.

Contact Number:
_____

Email: _____

Appendix 3: Presentations and publications arising from this study

- Presentations
- South African Equine Veterinary Association Congress, February 20-23 2020, Skukuza, South Africa
- European College of Veterinary Ophthalmology congress, May 28-31 2020, Rhodes, Greece
- International Equine Ophthalmology Consortium, June 11-13 2020, Lucerne Switzerland

- Publications
- The prevalence of ocular pathology in Warmblood horses in South Africa
- The effect of age on focal chorioretinopathy lesions in Warmblood horses