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Intravitreal injection of low-dose Gentamicin: an alternative method of management for equine recurrent uveitis

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Dedicated to Dr. med. vet. Richard McMullen Jr.

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LIST OF ABBREVIATIONS

AH	aqueous humor
cRALBP	cellular retinaldehyde-binding protein
CsA	cyclosporine
CSI	cyclosporine implant
DHODH	dihydroorotate dehydrogenase
ERU	equine recurrent uveitis
IMMK	immune mediated keratitis
IRBP	interphotoreceptor retinoid binding protein
IOP	intraocular pressure
IVG	intravitreal gentamicin
IVGI	intravitreal gentamicin injection
IVTA	Intravitreal triamcinolone injections
L	Leptospira
LP	leopard complex spotting locus
MAT	microagglutination test
MHC	major histocompatibility complex
PCR	polymerase chain reaction
PPV	pars plana vitrectomy
S	serum
SCI	suprachoroidal cyclosporine implant
TA	triamcinolone
UK	United Kingdom

1 Introduction

Equine recurrent uveitis (ERU), also known as moon blindness, recurrent iridocyclitis and periodic ophthalmia, affects up to 10% of the horses worldwide, and is widely recognized to be a complicated multifaceted disease (REBHUN 1979, SCHWINK 1992, DEEG et al. 2002). The disease is characterized by multiple recurrent bouts of inflammation interrupted by phases of quiescence, which may ultimately lead to blindness (BARNETT 1987, DEEG et al. 2002, GERDING and GILGER 2016, ALLBAUGH 2017). Clinical signs vary dramatically both in number, and intensity. In many instances, these clinical signs remain undetected by the owner. According to recent studies from Canada and the Southeastern United States, approximately one third of the horses evaluated were bilaterally blind at their first ophthalmologic examination (GERDING and GILGER 2016, SANDMEYER et al. 2017). These results demonstrate the need for early referral in cases diagnosed with ERU, even if the recognizable signs are mild. Blindness may lead to a horse being retired early, and in some instances may require euthanasia. Both situations are coupled with emotional, as well as financial concerns for their owners. (REBHUN 1979, ABRAMS 1990, GERDING and GILGER 2016).

Topical and/or systemic medical (immunosuppressive and anti-inflammatory) therapy is the foundation of any treatment protocol for horses affected with ERU. However, medical therapy alone cannot sufficiently control or suppress acute inflammation or prevent recurrent bouts of intraocular inflammation. In such instances, surgical intervention may be indicated (SPIESS 2010, ALLBAUGH 2017). Pars plana vitrectomy (PPV) and suprachoroidal cyclosporine implant (SCI) placement are both recognized as effective surgical treatment options for ERU (WERRY and GERHARDS 1992, WINTERBERG and GERHARDS 1997, FRÜHAUF et al. 1998, VON BORSTEL et al. 2005, GILGER 2010, GILGER et al. 2010, TÖMÖRDY et al. 2010).

More recently, minimally invasive treatment options such as intravitreal or suprachoroidal injections with triamcinolone (TA) have become more popular for the treatment of uveitis in both human and veterinary medicine (DEGENRING et al. 2003, BAATH et al. 2007, YI et al. 2008, GILGER et al. 2013a). These injection techniques are useful at suppressing acute inflammation, but are less effective at controlling the disease long-term or at preventing recurrences. PINARD (2005), followed by KLEINPETER (2014), MCMULLEN (2016), and LAUNOIS et al. (2019) first presented case series of horses affected with ERU that were successfully treated with low-dose

intravitreal Gentamicin injection (IVGI). With the exception of LAUNOIS et al. (2019), who used 6mg, the other case series were performed with 4mg gentamicin. The impetus for this treatment method was the PPV, as the irrigation solution contains 0.2-0.4 mg/ml of Gentamicin (WERRY and GERHARDS 1992, FRÜHAUF et al. 1998, WOLLANKE et al. 2001, VON BORSTEL et al. 2005). This led to speculation that treatment with low-dose intravitreal gentamicin injection may effectively suppress inflammation associated with ERU (PINARD 2005).

The anecdotal use of gentamicin is widely recognized, however, no studies evaluating the efficacy of this treatment method, or the potential complications associated with its use have been published.

The purpose of this study was to describe the intravitreal injection technique in the standing, sedated horse; to describe potential peri- and post-injection complications associated with the procedure, and to evaluate the ability of intravitreal gentamicin to control ERU.

2 LITERATURE OVERVIEW

2.1 Etiology and pathogenesis

2.1.1 Proposed etiologies

The earliest speculation about potential causes of ERU surfaced during the fourth century AD, when Vegetius hypothesised that the moon phases are associated with the recurrent nature of the disease, which then lead to the term ‘moon blindness’ (WERRY and GERHARDS 1992). Since then, multiple authors have investigated whether neoplasia, trauma, infectious organisms, auto-immunity and other miscellaneous factors are associated with the onset of clinical signs of uveitis (REBHUN 1979, MILLER et al. 1987, ABRAMS et al. 1990, SCHWINK 1992, GRAHN et al. 2000, MAGGS 2003, BLOGG et al. 2010, BRAGA et al. 2011, PRIEST et al. 2012).

Although the pathophysiology is not fully understood, ERU is considered an autoimmune disease. Its onset and severity is also associated with environmental factors and or genetic predisposition (MAIR and CRISPIN 1989, DAVIDSON 1992, ROMEIKE et al. 1998, KALSOW and DWYER 1998, DEEG et al. 2004 and Bellone 2017)) . Further supporting this thesis is the recurrent nature of the disease and the response of ERU to corticosteroids. Conversely, horses with ERU do not generally demonstrate a positive treatment response to antibiotics, which would be expected in

the case of a simple bacterial infection (MAIR and CRISPIN 1989, DAVIDSON 1992, ROMEIKE et al. 1998, DEEG et al. 2008). Furthermore, ERU shows many similarities to autoimmune uveitis in humans in terms of the relapsing-remitting course and the attack on the retina by T cells (KALSOW and DWYER 1998, WILDNER and KAUFMANN 2013).

2.1.2 ERU: an immune mediated disease

The predominant inflammatory cells in ERU affected eyes are CD4+ and T-lymphocytes, interleukin 2 and interferon-γ. Therefore, ERU is more specifically considered a T-helper type 1- mediated disease (ROMEIKE et al. 1998, GILGER et al. 1999, DEEG et al. 2002, DEEG et al. 2006). Although the precise mechanisms leading to initial and subsequent bouts of inflammation (i.e., recurrences) is not fully understood, it is commonly accepted that ERU develops following an initial bout of inflammation resulting in disruption of the blood ocular barrier (DEEG et al. 2002). Following disruption of the local immunity, CD4+ and T-lymphocytes gain access the eye, where they remain (ROMEIKE et al. 1998,GILGER et al. 1999, DEEG et al. 2001, DEEG et al. 2002 and DEEG et al. 2006).

The S-antigen, a photoreceptor protein found in rods and in the pineal gland and the Interphotoreceptor retinoid binding protein (IRBP), a large glycoprotein known to bind retinoids, were the first two autoantigens shown to cause an immune response (DEEG et al. 2001). Following identification of these antigens, IRBP was used to repeatedly induce uveitis in horses (DEEG et al. 2002). Thus, IRBP has been determined to be the major autoantigen responsible for the development of ERU (DEEG et al. 2002). The S-antigen, a major autoantigen in human medicine, resulted in a significantly lower number of uveitis cases when used experimentally to induce uveitis in horses (DEEG et al. 2004). Two additional retinal autoantigens have been detected more recently: Cellular retinaldehyde-binding protein (cRALBP), part of the rod and cone visual cycle, and synaptotagmin-1, a protein integral to synaptic vesicle membranes (DEEG et al. 2006, SWADZBA et al. 2012). DEEG et al. (2002) revealed that disease severity, as well as the T-cell response, varied amongst the uveitis cases following experimental induction with IRBP. These variations were attributed to different epitopes on the autoantigens being recognized (DEEG et al. 2002). This phenomenon was also observed in horses spontaneously affected by ERU (DEEG et al. 2002). This, so-called ‘epitope spreading’, is also thought to be responsible for the transformation of anterior

uveitis to a posterior uveitis, where new epitopes are exposed as a result of previous tissue destruction related to ocular inflammation (DEEG et al. 2001, DEEG et al. 2006). The specific targeting of such epitopes leads to the relapsing or recurrent nature of the disease (DEEG et al. 2001, DEEG et al. 2006). Despite prior identification of these autoantigens as well as the recognition of the consequent Type 1 T-helper cell-associated immune response, the molecular processes responsible for tissue destruction and secondary blindness are still not fully understood (GILGER and HOLLINGSWORTH 2016).

DEEG et al. (2007) showed, that the retinal autoantigens remain static in number even after severe destruction of the retina due to ERU. With these immune response triggering factors still present, recurrent inflammation may still develop even in non-visual or phthisical eyes (DEEG et al. 2007).

2.2 Leptospirosis and ERU

RIMPAU (1947) and HEUSSER (1948) first proposed an association between leptospirosis and ERU in the 1940s. After reports describing outbreaks of systemic leptospirosis consequently followed by high incidences of ERU (ROBERTS 1971), MORTER et al. (1969) were successful in repeating the described scenario experimentally. Following subcutaneous injections of blood containing *Leptospira* (L.) *interrogans* in a group of ponies, the herd developed signs of systemic leptospirosis, followed by ocular inflammation that developed weeks to months later (Morter et al. 1969, ROBERTS 1971).

Leptospirosis is a bacterial zoonotic disease, caused by one of more than twelve pathogenic species of *Leptopsira* (ADLER et al.2010). *L. Interrogans* and *L. petersenii* however, are the ones mostly associated with disease (ADLER et al.2010). The Gram negative spirochetes may mechanically penetrate mucous membranes or injured skin, thus gaining access to the vascular system where it can access many internal organs (MORTER et al. 1969). Most systemic infections are subclinical (HOUWERS et al. 2011 and BÅVERUD et al. 2009). However, seroprevalence, especially in older horses, is high (HOUWERS et al. 2011 and BÅVERUD et al. 2009).

Despite ERU being widely considered an immune mediated disease, *Leptospirosis* in the form of a persistent bacterial infection, is frequently considered an underlying cause of ERU (WOLLANKE et al. 2001, HARTSKEERL et al. 2004, BRANDES et al. 2007). However, neither specific antibiotic therapies nor vaccination contribute or result in

cessation of inflammation associated with ERU (KALSOW and DWYER 1998, ROHRBACH et al. 2005). Therefore, the infectious aetiology of *Leptospira* and resulting persistent bacterial infection is unlikely (KALSOW and DWYER 1998, ROHRBACH et al. 2005). The exact mechanism of induction of uveitis and the pathogenesis of how the bacteria is associated with the recurrences is not completely understood (GILGER et al. 2008, GILGER 2010). Increased intraocular antibodies compared to the serum, is indicative of local ocular antibody production (GOLDMANN and WITMER 1954). Recent studies support the 'molecular mimicry' hypothesis, with the immunogenic potential of certain components of the bacteria being integral to the development of ERU (PARMA et al. 1985, PARMA et al. 1987, PARMA et al. 1992, VERMA et al. 2010, VERMA et al. 2012). As such, *Leptospira* may account for the initial blood-ocular barrier disruption, which then triggers the subsequent immune reaction (REGAN et al. 2012).

Several studies investigating the prevalence of *Leptospira* organisms or associated antibody titers in serum and/or ocular fluids have been undertaken (BARNETT 1987, HALLIWELL et al. 1985, DWYER et al.1995, FABER et al. 2000, WOLLANKE et al. 2001, HARTSKEERL et al. 2004, GILGER et al. 2008, LOWE 2010, VANBorstel et al. 2010, MALALANA et al. 2017 and SAUVAGE et al. 2018). WOLLANKE et al. (2001) isolated *L. interrogans* from vitreous samples of 52% (120/229) of the horses undergoing pars plana vitrectomy as treatment for ERU in Southern Germany. Serum antibody titers ≥1:400 against one or more serovars of *L. interrogans* were found in 44% (106/241) of the horses and in the vitreous of 80% (194/242) of the horses, respectively (WOLLANKE et al. 2001). The most commonly isolated serovar was *L. grippothyphosa* (WOLLANKE et al. 2001). HARTSKEERL et al. (2004) isolated *Leptospira* in 32.2% (199/618) of the vitreous- and aqueous humor (AH) samples in their study. There was no distinction between the two ocular locations. In the studies carried out in North America, *L. pomona* was the most commonly isolated serovar HALLIWELL et al. (1985), DWYER et al. (1995) and FABER et al. (2000). FABER et al. (2000) used polymerase chain reaction (PCR) (21/30 horses, 70%) and culture (6/27 horses, 22.2%) to detect *Leptospira* in the aqueous humor and microagglutination test (MAT) for the evaluation of *Leptospira* antibody titers (positive titer ≥1:100) in the serum (24/28 horses, 85.7%). In contrast to the high prevalence of *Leptospira* in the previously described studies, GILGER et al. (2008) failed to detect any bacterial DNA in samples collected from ERU affected horses from the

Southeastern United States. Fifty-seven percent (57/100) of the vitreous humor samples had antibody titers against *Leptospira* VAN BORSTEL et al. (2010). A definition concerning the cut-off for a positive titer was not given. DNA was detected via PCR in 40% (40/100) of the samples from horses from Northern Germany VAN BORSTEL et al. (2010). The most prevalent serovar isolated in this study was *L. grippotyphosa* VAN BORSTEL et al. (2010). SAUVAGE et al. (2018) isolated *Leptospira* DNA in 30.3% (20/66) of the aqueous humor and/or vitreous samples via PCR, however, MAT was not performed.

In order to determine if local, intraocular antibodies against Leptospira are being produced it is recommended to calculate the ratio between the aqueous humor antibody titer and the serum antibody titer (GOLDMANN and WITMER 1954, GILGER 2008). The result, also known as the *Leptospira*-status of the eye, or the C-value, is considered positive, when the ratio is at least four (i.e., the aqueous humor antibody titer is at least four-fold higher than the antibody titer found in the serum) (GOLDMANN and WITMER 1954, GILGER 2008). The *Leptospira*-status is considered suspicious in the event that the value is at least one, but less than four (GILGER et al. 2008). A C-value less than one is considered negative (GILGER et al. 2008). GROOT-MINJES et al. (2006) determined that PCR results are an unreliable method of detecting the presence of *Leptospira,* as nearly 50% of the *Leptospira*-associated uveitis cases evaluated would have been missed using PCR alone.

Horses from the United Kingdom (UK) are not only less likely to be diagnosed with ERU, *Leptospira* also plays only a minor role in that geographic area (BARNETT 1987, LOWE 2010). MALALANA et al. (2017) recently investigated the role of *Leptospira* in the UK and found that only two of 30 ERU affected eyes had at least a four-fold increase of *Leptospira* aqueuous humor antibody titers when comparing aqueous humor to serum antibody titers. The most commonly isolated serovars in this study were *L. bratislava* followed by *L. autumnalis* MALALANA et al. (2017).

2.2.1 Genetic predisposition for ERU

It is suspected that genetics play an important role in the development and severity of ERU. Early speculations were triggered, when ERU rates decreased tremendously in the UK after stallions with cataracts were excluded from breeding (LORBEER 1940). By confirming that the MHC (major histocompatibility complex) gene region contributes

to a horse's risk of developing ERU, DEEG et al. (2004) provided evidence linking genetics and ERU.

Studies investigating the genetic predisposition of certain breeds, have shown that Appaloosas are eight times more likely to develop uveitis, and have a 3.8 times higher susceptibility to become blind from ERU than non-Appaloosas (DWYER et al. 1995, ANGELOS et al. 2009). Appaloosas are bred with a selection for the leopard complex spotting locus (LP) (FRITZ et al. 2014). The LP is incompletely dominant and most Appaloosas carry either a homozygous or heterozygous form (SPONENBERG et al. 2017). The LP is responsible for the breed's typical appearance with a distinct white pattern, which may have oval, pigmented spots within that white area (SPONENBERG et al. 2017). Homozygous horses for LP have the highest risk of developing ERU, thus, coat color may be used as a means to identify horses with the greatest risk (FRITZ et al. 2014). In addition to the LP-associated allele, two additional alleles on two different microsatellites, have been associated with an increased risk of ERU (FRITZ et al. 2014). However, the LP test is currently the only routine genetic test available (BELLONE 2017).

KUHLBROCK et al. (2013) carried out a genome-wide association study to identify potential risk loci conferring to ERU in German warmblood horses. The study revealed that genes Interleukin-17A and Interleukin-17F appeared to play an important role in the development of ERU, but further studies are required to test for influencing factors among genetic variants.

2.3 Definition of ERU

2.3.1 Classification and syndromes

Historically, ERU has been divided into the following syndromes: Classic ERU, insidious ERU, and posterior ERU (GILGER and MICHAU 2004). Classic ERU is characterized by inflammation of the uvea in association with other parts of the globe including the cornea, anterior chamber, lens, vitreous and retina, but which interrupted by variable phases of quiescence. The severity of the active bouts of inflammation tend to increase over time (REBHUN 1979, ABRAMS et al.1990, SCHWINK 1992). Insidious uveitis is difficult for the owner to recognize, as horses do not tend to show obvious signs of discomfort even in the face of severe intraocular inflammation (GILGER 2010, GILGER et al. 2004). A persistent low-grade inflammation is typical for this syndrome (GILGER 2010, GILGER et al. 2004). Appaloosas and draught breeds

tend to be overrepresented in the insidious ERU category (GILGER 2010, GILGER et all. 2004). Posterior uveitis mainly affects the posterior segment and is generally confined to the vitreous, retina and choroid (Schwink 1992). Therefore, this syndrome, which mostly affects Warmbloods, draught breeds and European horses, can likewise cause tremendous damage to the eye before being noticed by the owner (GILGER 2010, ALLBAUGH 2017).

2.3.2 Clinical symptoms

As the name suggests, ERU is characterized by recurrent inflammation of the uvea, which may involve the iris, the ciliary body or the choroid individually, or collectively (REBHUN 1979, MILLER and WHITLEY 1987, ABRAMS and BROOKS 1990, SCHWINK 1992, DAVIDSON 1992, GILGER and MICHAU 2004). Clinical signs may vary significantly in both quantity and intensity, and can include any combination of the following: photophobia, blepharospasm, enophthalmos, hypotony, conjunctival or episcleral vascular congestion, corneal edema, corneal vascularization, aqueous flare, hypopyon, hyphema, miosis, iris edema or congestion, , synechiae (anterior or posterior), cataract, vitreal traction bands, vitreal haze, depigmentation of the peripapillary region in a focal or alar pattern, loss of peripapillary fundic detail, retinal vascular congestion or edema, retinal cellular infiltrate (REBHUN 1979, MILLER and WHITLEY 1987, ABRAMS and BROOKS 1990, SCHWINK 1992, DAVIDSON 1992, GILGER and MICHAU 2004).

Signs commonly associated with ERU may be identified in association with other diseases such as secondary glaucoma, immune mediated keratitis (IMMK) or conjunctivitis, conjunctival foreign bodies, corneal ulcers or stromal abscesses (ALLBAUGH 2017). Establishing a correct and accurate diagnosis may present a significant challenge. This is especially challenging in other diseases with a persistent or recurrent pattern of inflammation, such as heterochromic iridocyclitis and keratitis (PINTO et al. 2014) and IMMK (REBHUN 1979, GILGER 2010).

2.4 Diagnostic testing for ERU *(Leptospira)*

2.4.1 Sample collection (aqueous humor, vitreous humor, serum)

Aqueous humor can be aspirated from the anterior chamber via aqueous paracentesis. This can be performed under general anaesthesia or under standing sedation with topical anaesthesia (FEATHERSTONE et al. 2013). It is crucial to aseptically prepare

the conjunctival fornices with 1.0 mL of dilute baby shampoo solution, 1.0 mL of a 1% dilute iodine solution, followed by 1.0 mL of balanced salt solution or eyewash prior to aqueous paracentesis (BROOKS et al. 2017.). Globe exposure may be facilitated using an eyelid retractor (FISCHER et al. 2019). The injection site of the left and right eyes are at the 1:00 and 11:00 o' clock position, respectively. A 27-30 gauge needle syringe combination or an insulin syringe with a swaged-on needle can be used to slowly aspirate 0.2-1.0 mL of aqueous humor (FEATHERSTONE et al. 2013, FISCHER et al. 2019). Historically, vitreous samples have mostly been collected during pars plana vitrectomy (WOLLANKE et al. 2001, HARTSKEERL et al. 2004, BRANDES et al. 2007, VON BORSTEL et al. 2010, BAAKE et al.2016). The injection site for vitreous paracentesis is located 10-12 mm from the dorsolateral limbus, in order to facilitate the needle placement through the pars plana and to placement of the needle through the sensory retina (MILLER et al. 2001). A 23- to 25 gauge needle is inserted through the sclera with the needle tip directed vitread towards the optic nerve (MILLER et al. 2001).

Serum can be obtained via routine blood draw from the jugular vein.

2.4.2 Methodology

Leptospira can be detected indirectly via microagglutionation test (MAT) or directly via polymerase chain reaction (PCR) or culture (MOCHMANN 1963). Concerning the direct methods, a positive test result is considered proof of *Leptospira's presence*, whereas a negative test result may be a false negative (MOCHMANN 1963). Taking a sample during a period of quiescence or failure to aspirate an adequate number of organisms may lead to a negative test result (MOCHMANN 1963).

2.4.2.1 Microagglution test (MAT)

With its high sensitivity and specificity, MAT is an effective way of testing samples for *Leptospira* (COLE 1973, BABUDIERI 1961, FAINE 1982). The samples are incubated with a live antigen and diluted up until 50% of the *Leptospira* are being agglutinated (GUSSENHOVEN et al. 1997, FAINE et al. 1999, LEVETT 2001). A titer of 1:100 or higher is considered positive, depending on the laboratory (AHMAD et al. 2005, OIE 2008). Comparing the antibody production of an intraocular compartment (aqueous humor/vitreous humor) to that of the serum, helps to detect the presence of and determine the severity of the local antibody production in the eye. A 4-fold increase,

when comparing the two localisations is considered positive (GOLDMANN and WITMER 2010).

2.4.2.2 Polymerase chain reaction (PCR)

PCR is a direct method to amplify specific DNA sequences of *Leptospira* with a high sensitivity and specificity (SMITH 1994, WIESNER e al. 1994). Another positive aspect is the relatively small amount of fluid (50-100 µL) that is needed to perform a PCR (SMITH 1994). It is not possible to determine the specific *Leptospira* serovar via PCR (AHMAD et al. 1995).

2.4.2.3 Cultures

Liquid or semi liquid culture media can be used for culture of *Leptospira* (BABUDIERI 1961). Currently, the most commonly used medium is EMJH base and enrichment medium, also referred to as bovine serum albumin-Twee 80 (LEVETT 2016). Cultures must be consistently evaluated for at least 16 weeks before they may be considered negative (ELLIS 1986).

2.5 Treatment of ERU

2.5.1 Medical management

The goals of treatment for ERU are the preservation of vision and the control of pain associated with intraocular inflammation (REBHUN 1979, MILLER and WHITLEY 1987, SCHWINK 1992, WERRY and GERHARDS 1992). Traditional medical management of ERU consists of topical and systemic medications to suppress and control inflammation and to facilitate pain management (non-steroidal anti-inflammatory drugs, corticosteroids, and mydriatics)(REBHUN 1979, ABRAMS and BROOKS 1990, WERRY and GERHARDS 1992). Topical administration of medications may not always be feasible. Horses that are experiencing significant ocular pain may be non-compliant, and present unique treatment challenges (YI et al. 2008). Each sequential bout of inflammation compounds the effects of the disease on the horse, which may ultimately lead to blindness (SPIESS 2010, GERDING et al. 2016, MCMULLEN et al.2017, SANDMEYER et al.2017). Therefore, not only is it crucial to suppress active inflammation, but also to prevent recurrent inflammation (GILGER et al. 2004, MCMULLEN et al. 2017). While medical management may be very effective at suppressing and controlling acute inflammation, it cannot effectively

prevent recurrent bouts of inflammation (GILGER et al. 2004, GERDING et al. 2016, MCMULLEN et al. 2017). For the long-term preservation of vision, early surgical intervention, if indicated, is essential (SPIESS 2010, ALLBAUGH 2017, MCMULLEN et al. 2017).

2.5.2 Intravitreal and suprachoroidal injections

2.5.2.1 Intravitreal rapamycin injections

Rapamycin is a carbocyclic lactone-lactam macrolide antibiotic (TREPANIER et al. 1998). Unlike tacrolimus and cyclosporine, rapamycin is a non-calcineurin inhibitor and promotes the expansion of T regulatory cells and inhibits the differentiation of pathogenic T helper 17 cells (KOPF et al. 2007). A clinical study published by DOUGLAS et al. (2008) showed that therapeutic concentrations of rapamycin were detectable for at least three weeks (the duration of the study) in the vitreous of normal horses following intravitreal injection. No intraocular toxicity was observed throughout the duration of the study (DOUGLAS et al. 2008). Studies on horses with naturally occurring ERU are required to evaluate the treatment outcome and complication rate associated with intravitreal rapamycin injections.

2.5.2.2 Intravitreal triamcinolone injections

Intravitreal injections with triamcinolone acetonide (IVTA) have been utilized in both human and veterinary medicine for the treatment of uveitis (DEGENRING et al. 2003, BAATH et al. 2007, YI et al. 2008). Triamcinolone acetonide, injected at a dosage of between 4 to 20 mg, lasts approximately 4 to 9 month in the vitreous of human patients (JONAS et al. 2006). A retrospective study in human medicine graded the IVTA as an exceptionally safe treatment method (BAATH et al. 2007). Caution is required in patients with a history of glaucoma, as an increase in intraocular pressure (IOP) is the most common complication associated with IVTA injections in people (BAATH et al. 2007). An additional glaucoma medication was required in 58.8% of the patients with pre-existing glaucoma (BAATH et al. 2007). In a study evaluating the ocular distribution and toxicity of triamcinolone acetonide following intravitreal injection in normal equine eyes, there was no report on the effect of TA on IOP (YI et al. 2008). The only complication reported following IVTA in normal equine eyes treated with 10, 20 or 40 mg of TA, was transient corneal edema, which was observed regardless of the drug

concentration used (YI et al. 2008). However, caution should be utilized when using IVTA in areas where fungal keratitis is overrepresented, as the high AH levels of TA in equine eyes following IVTA predispose these horses to fungal keratitis (YI et al. 2008). Following IVTA, triamcinolone acetonide cannot be removed and was associated with elevated ocular TA levels for the 21 day study duration (YI et al. 2008).
Three eyes from the control group and one eye from the treatment group developed endophthalmitis associated with bacterial infection, which emphasizes the need for proper aseptic preparation before any injection is performed (YI et al. 2008).

2.5.2.3 Suprachoroidal triamcinolone injections

The potential for complications associated with increased intraocular levels of TA, including a predisposition to fungal keratitis following IVTA can be largely avoided by injecting TA into the suprachoroidal space using custom-made micro needles (GILGER et al. 2013). A porcine model showed that suprachoroidal injection of TA (0.2mg and 2.0mg) had the same effect as IVTA (2.0mg) on controlling posterior uveitis (GILGER et al. 2013). Additionally, there were no adverse side effects noted, nor were any toxic reactions evident following suprachoroidal injections of TA (GILGER et al. 2013).

2.5.2.4 Low-dose intravitreal gentamicin injections

Gentamicin is a bactericidal aminoglycoside antibiotic that has been routinely added to the irrigation solution (0.2 – 0.4 mg/ml) during pars plana vitrectomy (WERRY and GERHARDS 1992, FRÜHAUF et al. 1998, VON BORSTEL et al. 2005, WOLLANKE et al. 2001). Based on this use, and the desire to avoid invasive intraocular surgery, it was utilized as a sole treatment option by injecting directly into the vitreous of horses with ERU (PINARD 2005). PINARD (2005) presented the first results from a group of horses treated with low-dose (4mg) IVGI. Eighteen eyes from ten horses were treated with IVGI under general anaesthesia (PINARD 2005). Seventeen of the 18 eyes treated with IVGI remained free from recurrences (PINARD 2005). KLEINPETER et al. (2014) presented data from 60 eyes treated with IVGI under general anaesthesia. Ninety-three percent of the eyes did not develop recurrences, but 11/60 eyes developed a cataract following IVGI (KLEINPETER et al. 2014). MCMULLEN (2015) presented the first case series of horses treated via IVGI under standing sedation. Thirty-three eyes were treated with 4mg IVGI and showed signs of improvement within 24 to 48 hours after the injection (MCMULLEN 2015). FISCHER et al. (2019) presented

data from 86 horses treated with IVGI (4mg) under standing sedation. A total of 88.1% of the horses remained free from persistent or recurrent bouts of inflammation following IVGI. Cataract formation was seen in 8.5% and retinal degeneration in 5.1% of the cases (FISCHER et al. 2019). Kleinpeter et al. (2019) later published the data from his presentation in 2014, with sixty-one treated eyes between the years of 2006 - 2013. Ninety-one point eight percent of these eyes had no further recurrences. Eighteen of the 61 eyes became blind, which was mostly due to cataract progression of a pre-existing cataract prior to IVGI. KLEINPETER et al. (2019) concluded that IVGI has a low complication rate and serves as a viable alternative to the PPV, a surgery widely utilized throughout Germany. LAUNOIS et al. (2019) presented a case series of 71 horses that were treated with 6mg IVGI. According to a telephone enquiry, 70/71 horses did not have another observable bout of inflammation within six month of the IVGI (LAUNOIS et al. 2019).

2.5.3 Surgical procedures

2.5.3.1 Suprachoroidal cyclosporine implants

Surgical intervention via cyclosporine (CsA) suprachoroidal implants (CSI) for the treatment of ERU was first published by GILGER et al. (2006). The mechanism of action of CSI is to inhibit calcineurin and therefore block Interleukin-2 transcription, which subsequently leads to an impaired proliferation of activated T-helper and T-cytotoxic cells (KERMANI-ARAB et al. 1985, KAY 1989, HOLLÄNDER et al. 1994). Horses with ERU that received a CSI had a significantly decreased frequency of uveitis flare-ups following surgery than before the implantation (GILGER et al. 2006). Previous cyclosporine A implant devices to treat ERU were designed to be placed directly into the vitreous (GILGER et al. 2000). The intravitreal drug levels of cyclosporine A were determined to be too low to suppress inflammation and further development led to the suprachoroidal implants that are currently utilized (GILGER et al. 2000, GILGER et al. 2006).

Mild temporary conjunctival hyperaemia is a reported complication associated with SCI placement (GILGER et al. 2006). Eleven percent of the horses in a study evaluating the long-term results following placement of a CSI were found to be blind due to uncontrolled uveitis or glaucoma, cataract progression or retinal detachment (GILGER et al. 2010). Vision was preserved following placement of a CSI in nearly 80% of the eyes with a minimum mean follow-up time of 28 month (GILGER et al. 2010). The

authors of this study recommend replacing the CSI prior to 48 months following implantation, as both clinical and in-vitro observations suggest a depletion of drug release from the device around that time (GILGER et al. 2006, GILGER et al. 2010).
There are specific challenges associated with CSI, especially in Europe, that one must consider when developing a treatment plan for horses diagnosed with ERU. First, the CSI are neither commercially available, nor are they FDA approved. Their import to any European country is thus forbidden, with the exception of academic institutions that have been granted explicit permission to import the CSI within the framework of an ongoing and existing research project (MCMULLEN et al. 2017). Second, horses with uveitis that cannot be controlled with conventional medical therapy are considered poor surgical candidates for the CSI, and alternative treatment options should be considered (GILGER and MICHAU 2004, GILGER et al. 2010, ALLBAUGH 2017, MCMULLEN et al. 2017).

2.5.3.2 Pars plana vitrectomy

The first surgical intervention used to treat ERU, PPV was adapted from human ophthalmology in the late 1980's (WERRY and HONEGGER 1987). The goal of the PPV is to remove the central vitreous body in order to remove vitreal opacifications that may be interfering with vision, as well as to remove any inflammatory cells sequestered within the vitreous (WERRY and Gerhards 1992). WERRY and GERHARDS (1992) first published a case series of horses affected with ERU treated via PPV. According to communications with the primary veterinarians, the 9/10 horses that underwent PPV and were released from the clinic did not experience any further recurrent bouts of inflammation (WERRY and GERHARDS 1992). Observed complications associated with the PPV were postsurgical pain, fibrin accumulation in the anterior chamber, vitreal hemorrhage, and one horse was euthanized due to irreversible blindness resulting from a complete retinal detachment (WERRY and GERHARDS 1992). WINTERBERG and GERHARDS (1997) evaluated 43 eyes from horses undergoing vitrectomy for treatment of ERU. Despite only 1/43 eyes experiencing further bouts of recurrent inflammation, 12/43 eyes were non-visual during reevaluation and 14/43 eyes demonstrated deteriorated visual status (WINTERBERG and GERHARDS 1997). Vision loss or decreased vision was contributed to cataract formation or progression, complete or partial retinal detachment, vitreal opacification, secondary glaucoma and phthisis bulbi

(WINTERBERG and GERHARDS 1997). In another study, 38 eyes from 35 horses undergoing PPV for ERU, from the University of Hannover, Germany, were evaluated postoperatively (FRÜHAUF et al. 1998). Horses with phthisis bulbi, secondary glaucoma or retinal detachment were excluded from the study population, and the surgery was only performed during a period of quiescence (FRÜHAUF et al. 1998). Long-term follow up examinations were carried out by the authors in 27/38 eyes, with the remainder of the follow-up data communicated through the owners or local veterinarians (FRÜHAUF et al. 1998). No further recurrences were detected in 85% of the eyes, however, vision was compromised in 15% of the eyes due to recurrent inflammation or cataract progression (FRÜHAUF et al. 1998). Intraoperative complications or complications observed immediately after surgery, were intraoperative vitreal or intraocular hemorrhage, vitreal hemorrhage post-anesthetic recovery, slight subretinal hemorrhage and transient hypopyon (FRÜHAUF et al. 1998). TÖMÖRDY et al. (2010) investigated the outcome of PPV with regard to the *Leptospira* status of the eye and concluded that PPV is not associated with a high rate of success in eyes without intraocular antibodies against *Leptospira*, as 85.7% of the cases with negative aqueous humor MAT results went on to develop further bouts of inflammation postoperatively (TÖMÖRDY et al. 2010) . The rate of recurrence in eyes testing positive for antibodies against *Leptospira* however was only 17.5%, this difference was statistically significant (TÖMÖRDY et al. 2010).

3 PUBLICATIONS

3.1 Intravitreal injection of low-dose gentamicin for the treatment of recurrent or persistent uveitis in horses: Preliminary results.

Own contribution:

The following tasks and examinations which are part of the first publication, have been performed by myself

- Participation in the examination, surgery and re-evaluation of the clinical patients
- Collection of data
- Establishing useful categories for the evaluation of the outcome and complications of the treatment modality
- Analysis, statistical evaluation and expression of data in tables
- Writing of the manuscript and working on corrections

The tasks of the other authors of this publication were the aim of the study, the lead and support of the project (Prof. Dr. W. Brehm and Dr. R. McMullen), as well as the support during the statistical evaluation (Dr. S. Reese). Furthermore, the outcome of the study was discussed, and the establishment and correction of the manuscript was supported (Prof. Dr. W. Brehm and Dr. R. McMullen).

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Intravitreal injection of low-dose gentamicin for the treatment of recurrent or persistent uveitis in horses: Preliminary results.

Britta M. Fischer[1], Richard J. McMullen Jr[1*], Sven Reese[2] and Walter Brehm[3]

Abstract

Background: Despite appropriate medical therapy, many horses with equine recurrent uveitis continue to suffer from recurrent bouts of inflammation. Surgical intervention via the pars plana vitrectomy or suprachoroidal cyclosporine implant placement may control and/or prevent recurrences, however, these procedures may be contraindicated, unavailable, or declined by an owner. Thus, an effective adjunctive treatment option may help to improve the clinical outcomes in those situations. There are several anecdotal reports on the use of intravitreal gentamicin injections, but to date, no data evaluating the complication rate and/or treatment effect following this treatment have been published. Thus, the aim of this prospective study was to describe the intravitreal gentamicin injection technique, describe the associated peri-injection (within 24 h) and post-injection (30 to 780 days) complications, and to report the effects of the injection on the clinical signs of uveitis. Additionally, evaluation of the systemic and ocular *Leptospira*-status, and its effect on the treatment outcome was performed. A total of 86 horses of various ages, breeds, and gender presenting with recurrent or persistent uveitis were treated via intravitreal injection of 4 mg of undiluted gentamicin (0.04 ml, Genta 100, 100 mg/ml in 35 horses) or preservative-free gentamicin (0.05 ml, 80 mg/ml in 52 horses) under sedation and local anesthesia. All 86 horses were observed for immediate peri-injection and post-injection complications. Response to therapy was evaluated in 59 of the 86 horses (follow-up: 30 to 780 days).

Results: Peri-injection complications consisted of subconjunctival (26/86; 30.2%) or intracameral hemorrhage (4/86; 4.7%); both of which completely resolved within 5 days. Post-injection complications consisted of cataract formation/maturation (5/59 horses, 8.5%) and diffuse retinal degeneration (3/59 eyes 5.1%). The majority of horses 52/59 (88.1%) with a minimum follow-up period of 30 days were controlled (absence of recurrent or persistent inflammation) at their last recheck examination. Recurrent inflammation was documented in 5/59 (8.5%) horses and persistent inflammation was diagnosed in 2/59 (3.4%) horses.

Conclusions: Intravitreal injection of low-dose gentamicin shows promise at controlling different types and stages of uveitis. The ability of intravitreal injections of low-dose gentamicin (4 mg) to control persistent and recurrent inflammation warrants further investigation.

Keywords: Equine recurrent uveitis, Gentamicin, Intravitreal injection, Equine ophthalmology, *Leptospira*

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Background

Equine recurrent uveitis (ERU) is widely recognized as an immune-mediated disease characterized either by recurrent bouts of ocular inflammation separated by variable periods of quiescence (lack of detectable ocular signs associated with active inflammation) or low-grade, persistent inflammation [1–3]. The cornerstone of treatment for ERU consists of local immunosuppression or immune-modulation in conjunction with systemic anti-inflammatory treatment [4–7].

In addition to medical therapy, there are two widely utilized surgical procedures, cyclosporine suprachoroidal implants (CSI) and pars plana vitrectomy (PPV), that are routinely performed to treat horses with ERU [8–14]. Implantation of a CSI has been proven to be an effective means of controlling uveitis in horses responsive to prior medical therapy [3, 8, 13, 15]. However, because their legal importation into Europe is restricted to academic institutions for specific use in ongoing research, the use of CSI is severely limited on this continent. A more commonly performed surgery in Europe (especially Germany) is the PPV [9, 10, 14, 16]. Initially, PPV was utilized to treat all forms of ERU, but recently published data suggests that it is most effective in horses with confirmed leptospiral etiology [14].

Two recent studies demonstrated the relative inability of medical therapy to adequately control and prevent long-term complications and blindness in a large proportion of horses evaluated, highlighting the importance of additional treatment modalities [17, 18]. Intravitreal injections with triamcinolone acetonide or rapamycin have been successfully utilized in the management of recurrent uveitis in humans, as well as in small groups of horses [19–23]. However, the rate of complications and lack of long-term control of ERU, has limited their use in equine ophthalmology. Gentamicin (0.2–0.4 mg/ml), a bactericidal aminoglycoside antibiotic, has been routinely added to the PPV irrigation solution since the surgery's introduction in the early 1990s [9–11, 24]. This led to speculation that low-dose intravitreal gentamicin (4 mg) injections (IVGI) alone could serve as an alternative treatment for ERU, and initial results were presented by Pinard, et al. in 2005 [25]. Despite widespread anecdotal use, there are no published studies evaluating the efficacy of this treatment or establishing the risk of complications following IVGI.

The purpose of this prospective study was to describe the intravitreal gentamicin injection technique, to identify any peri-injection (within 24 h) and/or post-injection (30 to 780 days follow-up) complications associated with the IVGI and to evaluate the clinical outcome in horses with ERU, following a single 4 mg IVGI. Additionally, aqueous humor (AH) and serum (S) samples were evaluated for the presence of leptospiral antibody titers (S and AH) and leptospiral DNA (AH) and their effect on the treatment outcome.

Results

Horses

A total of 86 horses with a mean follow-up period of 165.9 ± 190.3 days (range: 1 to 780 days) were included in the present study. The mean age was 11.6 ± 5.5 years (range: 2 to 28 years). Gender, breed and coat color distribution can be found in Table 1. Twenty-nine horses were treated bilaterally, resulting in one eye being randomly selected for evaluation.

Fifty-nine of the 86 eyes had a minimum follow-up period of 30 days (range: 30 to 780 days) and comprised the group undergoing statistical evaluation of post-injection complications and clinical treatment outcome. Fifty-two of 59 eyes (88.1%) were controlled (non-recurrence/persistence, independent of complications) after the IVGI, and despite the discontinuation of topical and medical therapy. Overall, 5/59 eyes (8.5%) presented with recurrent and 2/59 eyes (3.4%) presented with persistent inflammation during follow-up examination. The follow-up data and corresponding results of positive outcome are listed in Table 2. Category distributions are listed in Fig. 1, Tables 3 and 4.

Leptospira status

Aqueous humor was obtained from 79/86 (91.9%) eyes and serum from 80/86 (93%) horses. Table 5 shows the calculated c-values for each individual *Leptospira* serovar and the combined results are documented in Table 6. *Leptospira* PCR was performed on 79 eyes, 23 of which were positive 23/79 (29.1%). Based on our inclusion criteria and calculation of c-values for each eye 50/79 eyes (63.3%) were classified as *Leptospira* negative, 13/79 eyes (16.5%) were classified as *Leptospira* suspicious and the remaining 16/79 eyes (20.3%) were classified as *Leptospira* positive.

Peri-injection complications

Subconjunctival and intracameral hemorrhage (due to the aqueous paracentesis) were seen in 26/86 (30.2%) and 4/86 (4.7%) of the eyes, respectively, but were completely resolved within 5 days.

Post-injection complications

Fifty-nine of 86 eyes had a minimum follow-up period of 30 days (30–780 days) and were evaluated for the presence of post-injection complications. Cataract formation/maturation was observed in 5/59 (8.5%) eyes, and retinal degeneration was seen in 3/59 eyes (5.1%). Four of the five cataracts that developed post-injection were identified in horses that received gentamicin with preservatives. Cataract progression/maturation occurred within

Table 1 Gender, breed and coat color distribution of the horses ($n = 86$) that had undergone IVGI between January 2013 – June 2016

Gender *n* = 86	Geldings	49	(57%)
	Mares	31	(36%)
	Stallions	6	(7%)
Breed n = 86	Warmblood	38	(44.2%)
	Quarter Horse; Paint Horse	9	(10.5%)
	Icelandic Horse	6	(7.0%)
	Pony	6	(7.0%)
	Heavy Warmblood	5	(5.8%)
	Standardbred Trotter	5	(5.8%)
	Haflinger	5	(5.8%)
	Appaloosa	4	(4.7%)
	Spanish	3	(3.5%)
	Knabstrupper	3	(3.5%)
	Thoroughbred	2	(2.3%)
Coat Color Distribution n = 86	Bay	36	(41.9%)
	Chestnut	16	(18.9%)
	Gray	11	(12.8%)
	Leopard-patterned	11	(12.8%)
	Black	7	(8.1%)
	Dun	5	(5.8%)

one week (1/5, 20%), within one year (3/5, 60%), and later than one year post-IVGI (1/5, 20%). All eyes, that developed mature cataracts, presented with cataracts of different stages before IVGI (Fig. 2). Retinal degeneration, not associated with obvious visual deficits (as assessed via menace response), was identified in 3/59 (5.1%) eyes (Fig. 3), and was consistently identified as a horizontal, geographic area (between one and three disc diameters in size) of diffuse tapetal hyperreflectivity superior to the optic nerve head. This complication was identified in a single eye at each of the following time points: Within 30 days, between 30 and 60 days, and between 90 and 122 days following IVGI, respectively. None of these eyes showed signs of retinal degeneration prior to IVGI.

Statistical evaluation of factors influencing treatment outcomes and complication rates

Variables with a significant effect on treatment outcome or on the development of long-term complications, are presented in Table 7. A significant correlation was identified between the Appaloosa breed and recurrence of inflammation ($P < 0.001$). In each of these horses, uveitis remained controlled in the early stages of follow-up. However, over time, aqueous flare was detectable in all three eyes. Leopard-patterned horses were more likely to develop recurrent inflammation than horses with other coat colors ($P = 0.049$). None of the eyes, that developed retinal degeneration, had detectable aqueous flare pre-IVGI ($P = 0.046$). The presence of subconjunctival or intracameral hemorrhage post-IVGI did not have a significant influence on either the control of the uveitis, or the development of long term-complications. Neither the clinical diagnosis, nor the additional categories utilized to subjectively grade equine uveitis in the present study had any influence on the control of the uveitis or the development of post-injection complications. The *Leptospira* antibody status of the eye (positive, suspicious, or negative C-value) and aqueous humor *Leptospira* PCR results did not have a significant influence on the development of long-term complications. There was a significant influence on the development of persistent inflammation in one eye with multiple positive C-values ≥4 (C-value of 8 for *L. pomona* and C-Value of 4 for *L. grippotyphosa*) ($P = 0.015$), multiple aqueous humor titers ≥1:400 (aqueous humor titer for *L. Pomona* 1:800 and for *L. grippotyphosa* 1:1600) ($P = 0.013$) and one positive serum titer ≥1:400 (serum titer for *L. grippotyphosa* 1:400) ($P = 0.047$). Although not significant, 4/5 (80%) mature cataracts developed following IVGI injection with gentamicin containing preservatives (Genta100).

Discussion

Many horses with ERU require additional treatment modalities in addition to medical therapy. Both the PPV and CSI placement are commonly performed in Europe and the USA, respectively [8, 15, 16, 26]. Although CSI placement can effectively suppress intraocular

Table 2 Follow-up periods and clinical outcomes post-intravitreal gentamicin injections

Minimum follow-up period	Controlled ERU (no recurrent or persistent inflammation, independent of complications)	Average follow-up period (days)	Standard deviation (± days)	Range (days)
30 days 59/86 (68.6%) eyes	52/59 eyes 88.1%	238	190	30–780
3 month 43/86 (50.0%) eyes	36/43 eyes 83.7%	313	175	93–780
5 month 34/86 (39.5%) eyes	27/34 eyes 79.4%	359	164	153–780
7 month 24/86 (27.9%) eyes	20/24 83.3%	407	170	213–780
1 year 12/86 (14.0%) eyes	9/12 eyes 75%	541	137	365–780

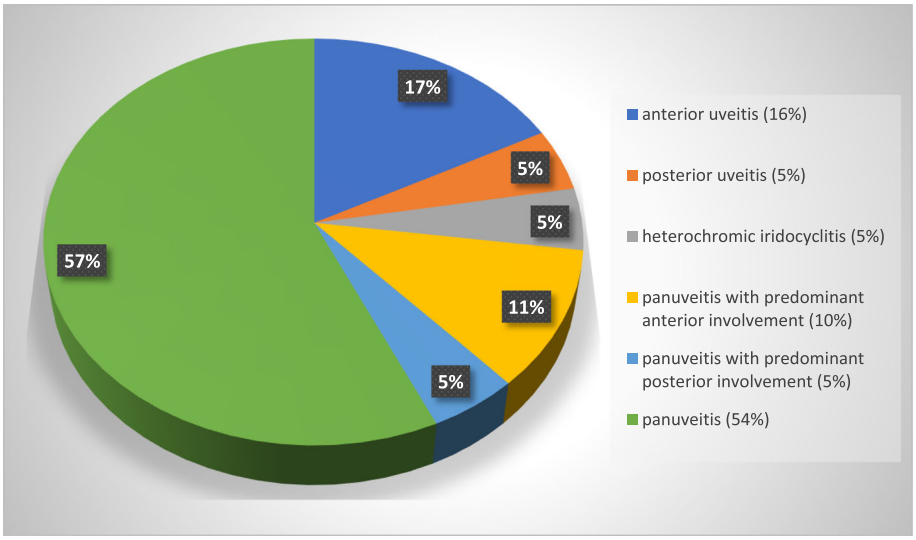

Fig. 1 Distribution of clinical signs of uveitis

inflammation and prevent recurrent inflammatory bouts for several years, they are not readily available in Europe due to legal restrictions governing their importation. Additionally, there are several instances where PPV (e.g., radial retinal detachments, late immature to mature cataracts present) or CSI (e.g., uncontrollable inflammation despite appropriate medical therapy) are contraindicated. Thus, additional or alternative treatment options, such as IVGI, may enable us to better control this highly debilitating disease. attractive.

Aqueous paracentesis and IVGI can be performed during the same sedation, using minimal regional and topical anesthesia; thus, negating the need for general anesthesia. Ultimately, only those horses not being controlled with IVGI require additional surgical intervention. This has dramatically reduced the number of horses requiring surgical intervention to control ERU in our clinic population. However, if an eye fails to be controlled with IVGI, the *Leptopsira* status, previously obtained via aqueous paracentesis, can then be used to choose the most appropriate surgical intervention, e.g., a PPV or CSI.

Serum *Leptospira* antibodies are able to effectively cross the blood-ocular barriers in the presence of uveitis, therefore only local antibody production at the site of the inflamed tissue is a true indicator for a *Leptospira*-induced mechanism of action. Individual aqueous humor or serum antibody titers are unreliable predictors of involvement [27]. In order to accurately identify *Leptospira's* role in the pathogenesis of equine uveitis it is important to calculate the c-value (i.e., the ratio between aqueous humor and serum antibody titers) [14].

The main goals in treating ERU are the reduction of ocular inflammation, the reduction or elimination of pain or discomfort, and the preservation of vision [3, 17]. According to a recent study by Gerding and Gilger, nearly half of the eyes affected with uveitis became blind, regardless of the therapy implemented [17]. In a study from Germany, evaluating the post-operative results following PPV for the treatment of ERU, 17/43 (39.5%) of the eyes had improved vision, 14/43 (32.6%) of the eyes demonstrated reduced vision, and 12/43 (27.9%) of the eyes were blind following the surgery [12]. Long-term results following implantation of a CSI revealed that 119/151 (78.8%) of the eyes remained visual [13]. Although the results of the present study (Table 4) are not directly comparable with the previous reports, the vision status of the eyes in this study remained unchanged following IVGI in 71.2% (42/59) eyes, were improved in 18.6% (11/59) eyes, and deteriorated in 10.2% (6/59) eyes. Each eye that developed mature cataracts post-IVGI had some degree of immature cataract maturation prior to IVGI (Fig. 2). Despite

Table 3 Characteristics of uveitis

acute/chronic n = 86 eyes	**acute** 4 (4.7%)		**chronic** 25 (29.1%)		**chronic-acute** 57 (66.3%)
recurrent/persistent n = 86 eyes		**recurrent** 36 (41.9%)		**persistent** 50 (58.1%)	
presence of aqueous flare n = 86 eyes	**0** (no flare) 42 (48.8%)	**1** (faint flare) 20 (23.3%)	**2** (moderate flare) 13 (15.1%)	**3** (severe flare) 3 (3.5%)	**4** (blood or fibrin in anterior chamber) 8 (9.3%)

Table 4 Subjective visual assessment pre- and post-IVGI and the subsequent change, or lack thereof, in the individual eyes evaluated

Pre-IVGI n = 86 eyes	**GOOD** 42 (48.8%)	**REDUCED** 20 (23.3%)	**POOR** 24 (27.9%)
Post-IVGI*n* = 59 eyes	**GOOD** 38 (64.4%)	**REDUCED** 8 (13.6%)	**POOR** 13 (22.0%)
Change in status following IVGI n = 59 eyes	**UNCHANGED** 42 (71.2%)	**IMPROVED*** 11 (18.6%)	**DETERIORATED**** 6 (10.2%)

*from a **POOR** to a **REDUCED** or **GOOD** vision status or from a **REDUCED** to a **GOOD** vision status)**from a **GOOD** to **REDUCED** or **POOR** or from **REDUCED** to **POOR** vision status due to cataract formation or phthisis bulbi

this, the degree of cataract maturation prior to IVGI cannot be reliably utilized to predict the likelihood of cataract progression/maturation following IVGI. None of the horses that developed post-IVGI retinal degeneration demonstrated any subjective behavioral changes (e.g., head carriage abnormalities (head tilt), spooky or erratic behavior, hesitant to move or navigate obstacles in a known environment) suggesting that vision was compromised, nor did they not show detectable clinical signs of vision loss (menace response). However, we cannot conclude that vision was not compromised. Additional functional testing methods, such as pre-IVGI and post-IVGI electroretinography (ERG) would provide more objective and meaningful results pertaining to retinal function, and should be considered for future studies. Although none of the three eyes that developed retinal degeneration had flare at the time of IVGI, the clinical disease progression in each of these horses differed significantly enough to prevent us from drawing a reliable conclusion as to why this complication occurred. We cannot exclude that eyes presenting with a mature cataract did not develop retinal degeneration. The risks of potential cataract maturation and retinal degeneration cannot be ignored, and must be discussed in detail with the owner when discussing treatment options. There are many factors that will ultimately determine if an IVGI is indicated, thus, it is important to provide an accurate risk-benefit analysis for each individual horse. Further investigation into the post-injection development of retinal degeneration is warranted. Ongoing efforts include functional testing via ERG and posterior segment evaluation via optical coherence tomography (OCT). The fact that there was a significant influence on the development of persistent inflammation in one eye with multiple positive C-values ≥4 of different *Leptospira* titers, multiple aqueous humor *Leptospira* titers ≥1:400 of different serovars and one positive serum *Leptospira* titer ≥1:400 warrants further investigation, but caution must be taken when interpreting this data, as only a single eye was affected. The complication rates associated with IVGI (88.1% non-recurrence/non-persistence rate, 8.5% cataract progression/maturation, and 5.1% retinal degeneration) are comparable to published results following CSI placement (46% non-recurrence rate, 16% cataract progression/formation and 16% retinal degeneration) and PPV (73.6–100% non-recurrence rate, 38.2–44.2% cataract progression/formation, and 9.3% retinal degeneration) [9–14].

Presently, the mechanism of action of gentamicin on the disease process in ERU and other types or stages of equine uveitis remains enigmatic. Positive suppression of inflammation, which can be observed as early as 24–48 h post-IVGI, was achieved in various types and stages of equine uveitis despite the *Leptospira* status of the eye, in the present study. Therefore, we speculate that rather than having a direct bactericidal effect on putative bacterial organisms, gentamicin instead influences or interferes with the immune-mediated processes intrinsic to ERU. Although purely speculative, the underlying mechanism of action of gentamicin may block or suppress the activation of specific T-cell lines; cells that are known to play a significant role in autoimmune uveitis [28]. Further research into gentamicin's mechanism of action following intravitreal injection is necessary.

Limitations of the present study are the short follow-up periods utilized for evaluation following IVGI. Despite the short follow-up duration, a 30-day minimum follow-up period was selected in order to capture the immediate effects of IVGI and to ensure that all complications seen associated with this technique were observed and recorded. Had we selected a longer minimum follow-up period, 1/3 (33.3%) of the eyes that

Table 5 Single c-value results for individual *Leptospira* serovars

Result	C-values for individual Leptospira (L.) serovars								
	sejroe (*n* = 63)	saxkoebing (n = 63)	canicola (*n* = 63)	autum-nalis (*n* = 79)	grippo-typhosa (*n* = 79)	pomona (*n* = 79)	australis (n = 79)	ictero-haemorrhagiae/ copenhageni (n = 79)	bratislava (n = 79)
Posititive (+)	**0**	**0**	**0**	**1** (1.3%)	**15** (19.0%)	**4** (5.1%)	**1** (1.3%)	**1** (1.3%)	**1** (1.3%)
Negative (–)	**63** (100%)	**62** (98.4%)	**63** (100%)	**74** (93.7%)	**54** (68.4%)	**69** (87.3%)	**75** (95.0%)	**77** (95.0%)	**75** (95.0%)
Suspicious (?)	**0**	**1** (1.6%)	**0**	**4** (5.1%)	**10** (12.7%)	**6** (7.6%)	**3** (3.8%)	**1** (1.3%)	**4** (5.1%)

Table 6 Combined serum and aqueous humor *Leptospira* titer results and the corresponding *Leptospira* c-value results

Titer	negative titer(s)	SINGLE SEROVAR positive titer (< 1:400)	MULTIPLE SEROVARS positive titers (1:100–1:400)	SINGLE SEROVAR positive titer (> 1:400)	MULTIPLE SEROVARS positive titers (> 1:400)
Serum (*n* = 80)	**41** (51.3%)	**20** (25.0%)	**10** (12.5%)	**6** (7.5%)	**3** (3.8%)
Aqueous humor (n = 79)	**49** (62.0%)	**11** (13.9%)	**1** (1.3%)	**13** (16.5%)	**5** (6.3%)
C-value (different serovars)	**NO positive c-value**	**SINGLE positive c-value** (less than 4)	**MULTIPLE positive c-values** (less than 4)	**SINGLE positive c-value** (greater than 4)	**MULTIPLE positive c-values** (greater than 4)
C-value (n = 79)	**50** (63.3%)	**12** (15.2%)	**1** (1.3%)	**11** (13.9%)	**5** (6.3%)

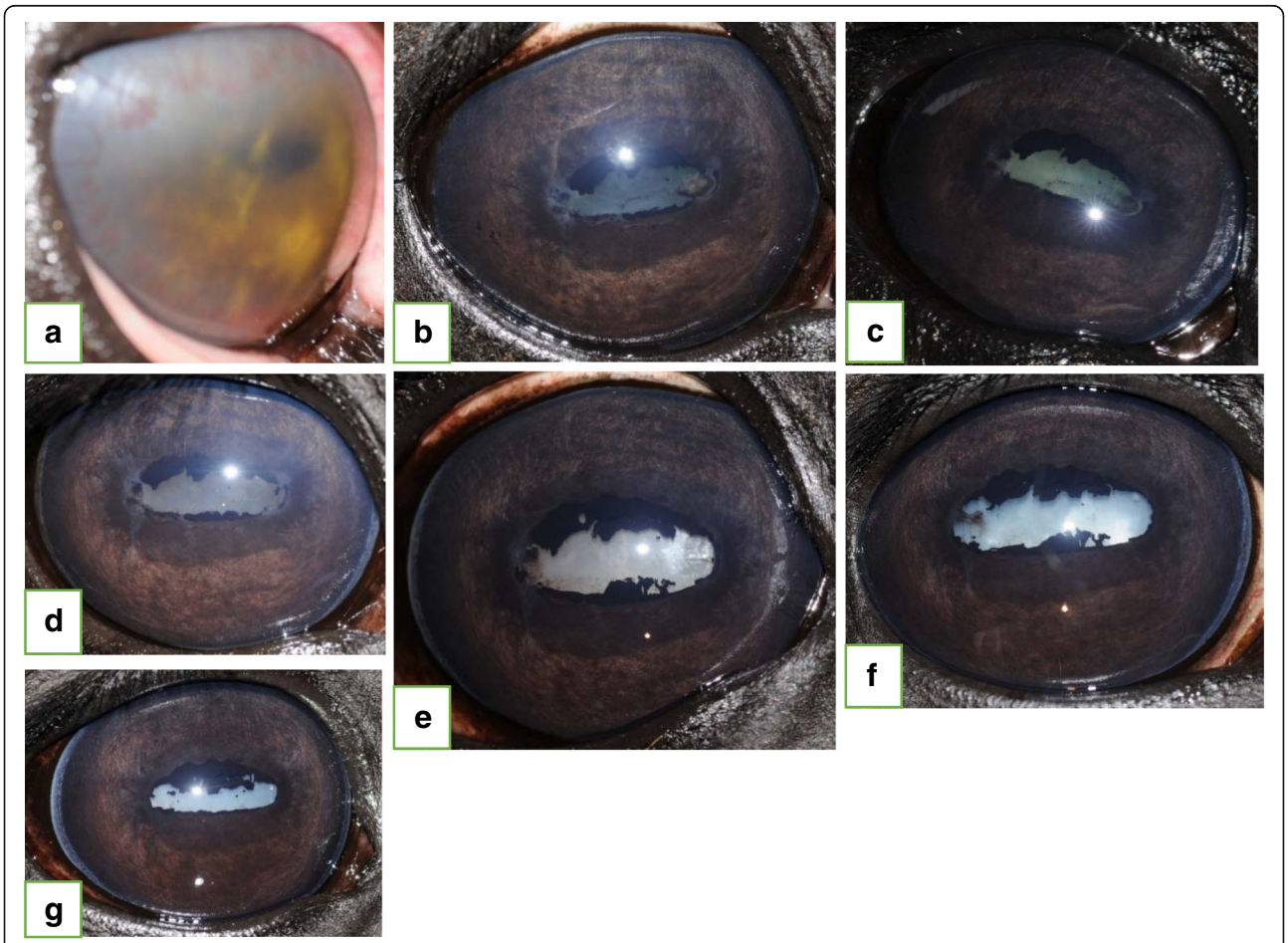

Fig. 2 Nine-year old warmblood gelding presented with chronic-acute, persistent panuveitis of the right eye (OD). Negative c-value for *Leptospira*. **a**. Initial presentation: Blepharospasm, epiphora, diffuse corneal edema, 360° corneal neovascularization, + 4/4 flare, fibrin in anterior chamber and miosis were present. The posterior aspect of the eye could not be visualized. Inflammation was controlled with medical therapy. **b.** One-hundred-fifteen days post-initial presentation: Two additional bouts of inflammation since initial presentation. Both intravitreal gentamicin injection (IVGI) and aqueocentesis were performed. Immature cataract and posterior synechia were present at the time of IVGI OD. **c**. Seven days post-IVGI OD: Immature cataract. **d.** Sixty days post-IVGI OD: Medical treatment was discontinued 39 days prior to this examination. No signs of active inflammation could be identified. **e.** One-hundred-thirteen days post IVGI OD: Mature cataract (cataract maturation). No signs of active inflammation. **f.** Three-hundred-twenty-seven days post-IVGI OD: Uveitis remains controlled without medical treatment. **g.** Six-hundred-two days post-IVGI OD: No recurrent bouts of inflammation since IVGI

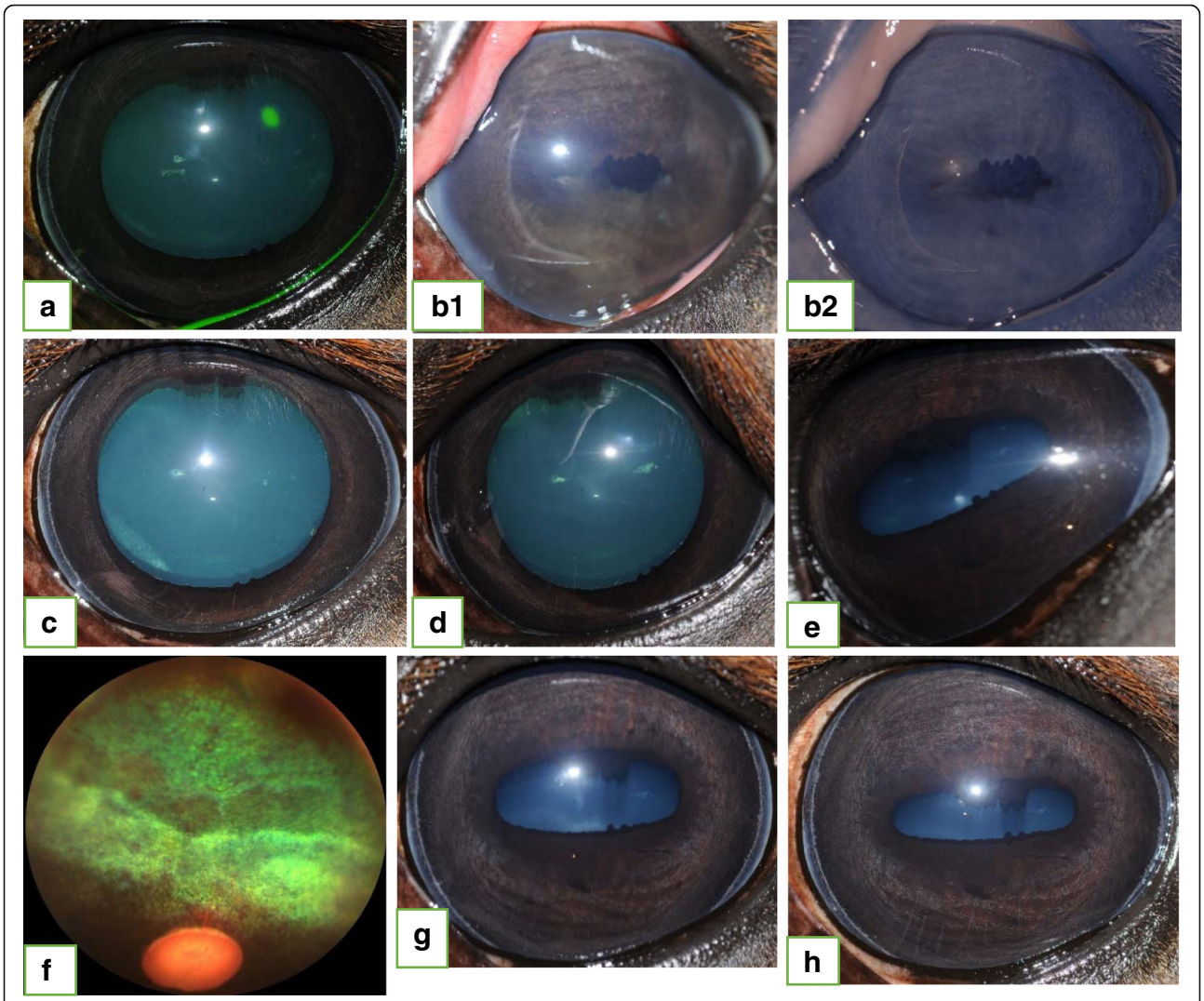

Fig. 3 Eight-year old warmblood mare that presented for chronic-acute, recurrent anterior uveitis in the left eye (OS). Negative c-value for *Leptospira*. **a.** Initial presentation: Two days following the onset of an acute bout of inflammation. Topical and systemic therapy were initiated by the referring veterinarian, when ocular signs were first identified. Inflammation was controlled via medical therapy. **b1.** Seven months after initial presentation: Active uveitis OS; + 4/4 flare, fibrin and complete miosis were present. **b2.** Infrared picture of OS at the same examination as in B1. **c.** Uveitis was controlled within 5 days of initiating medical therapy. **d.** Recurrent acute inflammation: 14 months later. Intravitreal gentamicin injection (IVGI) and aqueocentesis were performed. **e.** Ninety-eight days post-IVGI OS: Uveitis has remained controlled following IVGI. A focal area of tapetal hyperreflectivity identified during indirect ophthalmoscopy of the fundus. **f.** Fundus image of the lesion described in **e.** Retinal degeneration developed between the 30- and 98- day recheck examination. Subjective vision status unchanged from pre-IVGI. **g.** Two-hundred-seventy days post-IVGI OS: Uveitis controlled. Retinal degeneration remains static. **h.** Three-hundred-eighty-five days post-IVGI OS: Uveitis remains controlled. Retinal degeneration remains static

developed retinal degeneration and 1/5 (20%) of those, where cataract progression was observed would have not been included in our results, thus introducing a false positive bias into our complication rates. One cataract, that would not have been included otherwise, developed when using preservative-free gentamicin. Thus, setting the minimum-follow-up period at a time point further out from the IVGI (e.g., 5 months) would have prevented inclusion of those complications from our results, as a result, an incorrect conclusion would have been drawn that no cataract progression/maturation occurred when utilizing PFG for the IVGI. Future studies evaluating the clinical outcome and the presence or development of long-term complications over multiple years are necessary and are currently underway.

The present study reports control of ERU in 88.1% (non-recurrence/non-persistence rate) in the absence of medical treatment of the eyes with a minimum follow-up period of 30 days. These results support the

Table 7 Individual variables with positive correlation to treatment outcome or the development of post-injection complications

Variable	Treatment Outcome	Post-Injection Complications
Breed	Appaloosas: more recurrent inflammation than other breeds	NC
Coat color	Leopard patterned: more recurrent inflammation than other coat colours	NC
Flare pre-IVGI	NC	NO flare: increased risk of retinal degeneration
Leptospira status of the eye	Multiple positive C-values (≥4) for multiple individual serovars: increased incidence of persistent inflammation	NC
Aqeuous humor titer in total	Multiple positive individual aqueous humor titers for *Leptospira* (≥1:400): increased incidence of persistent inflammation	NC
Single serum titer	One positive serum titer for *Leptospira* (≥1:400): increased incidence of persistent inflammation	NC

Abbreviations: *NC* no correlation

anecdotal findings reported by Pinard in 2005 with a positive outcome of 94.4%, and those reported by Kleinpeter in 2014 showing a positive outcome in 93.3% (follow-up: 2–96 month) [25, 29]. In the latter study, 11/60 (18.3%) eyes became blind due to cataract formation following IVGI [25]. When comparing this result to the first 34 eyes in the present study that were treated with gentamicin containing preservatives, similar rates of cataract formation or maturation (4/34 (11.8%) eyes) were observed [25]. Because cataract maturation was observed most often in eyes with moderate pre-existing cataracts, we speculated that the preservatives in Genta 100 may have contributed to the accelerated cataract maturation in these cases. In order to minimize this risk, we switched to PFG solution after making this observation. After switching to PFG, only a single cataract progressed from immature to mature (1/52 eyes, 1.9%). Although the exact risk of cataract maturation associated with PFG IVGI is unknown, it appears that utilization of a PFG solution may help to minimize the actual risk of developing this blinding complication post-IVGI.

Conclusion

With less than 9% of the horses in the present study developing recurrent or persistent inflammation, less than 9% with cataract maturation and less than 6% with retinal degeneration, IVGI was associated with a lower level of complications compared with medical therapy [3, 17, 18] and other commonly implemented surgical treatment options for ERU (CSI placement and PPV) [10–13]. The ability of low-dose IVGI with 4 mg gentamicin (especially PFG) to suppress active inflammation in various types and stages of equine uveitis in the present study despite the *Leptospira* status of the eye, adds another treatment option in the management of a severely debilitating and vision threatening disease.

Methods

Case selection

Complete initial and all follow-up ophthalmic examinations were performed by a board-certified veterinary ophthalmologist (RJM) between January 2013 through June 2016 in south-east Germany. Horses presenting with signs of active or chronic uveitis and a history of recurrences were included in the study. Signs associated with recurrent or persistent uveitis included, but were not limited to, blepharospasm, epiphora, keratic precipitates (KP), aqueous flare, fibrin in the anterior chamber (AC), hyphema, miosis, corpora nigra atrophy or degeneration, iris hyper- or depigmentation, equatorial vesicular cataracts, posterior lens capsule adhesions or opacifications, vitreous body opacifications, and retinal detachment. Horses with uveitis resulting from putative trauma, secondary to infectious corneal diseases, or following intraocular surgery, were excluded.

Owners were educated on the various medical and surgical (i.e., PPV, CSI, and intravitreal injections) treatment options, and risks associated with each option. Client consent to perform the IVGI was obtained following an in-depth discussion of potential complications including failure of the selected treatment option to control the disease, resulting in persistent/ recurrent inflammation with progression of ocular signs, and potential cataract maturation or development and retinal degeneration or detachment.

Examination

Complete ophthalmic examinations were performed on initial presentation, and on each subsequent follow-up examination, and consisted of a subjective clinical vision assessment (menace response) and neuro-ophthalmic evaluation (dazzle, and pupillary light reflexes (PLR)), slit lamp bio microscopy (Kowa SL-15)',[1] indirect ophthalmoscopy (Keeler Vantage),[2] rebound tonometry (TonoVet),[3] external ocular fluorescein dye

application (Fluoreszein SE Thilo),[4] and color (Nikon D300s)[5] and infrared (Nikon D200)[5] (sensor conversion)[6] digital imaging. Aqueous flare was graded as follows: 0 (none), 1 (faint), 2 (moderate), 3 (severe) or 4 (blood or fibrin present in the anterior chamber) [30]. Fundus images (Kowa Genesis or ClearView)[1,7] were obtained in horses with posterior segment abnormalities, when possible.

Categorization of uveitis

For statistical purposes, each case was diagnosed with one of the following: 1. Panuveitis (global uveal inflammation with equal distribution of clinical signs between the anterior and posterior segment); (Figs. 2 and 4); 2. Panuveitis with predominant anterior segment involvement; 3. Panuveitis with predominant posterior segment involvement; 4. Anterior uveitis; (Fig. 3); 5. Posterior uveitis; and 6. Heterochromic iridocyclitis with secondary keratitis (HIK), a recently described, specific form of idiopathic anterior uveitis (iridocyclitis) and corneal endothelial inflammation associated with iris pigment dispersion and retro-corneal fibrous membrane formation [2, 3, 8, 31].

Each case was further categorized as **"acute"** (active inflammation without overt signs of chronicity), **"chronic"** (signs of chronicity but no signs of active inflammation) or **"acute/chronic"** (acute onset of inflammation associated with chronic inflammation). **"Recurrent uveitis"** was diagnosed when at least 2 episodes of recurrent inflammation occurred despite appropriate medical therapy leading to a period of quiescence following cessation of medical therapy. **"Persistent inflammation"** was diagnosed when an initial or recurrent bout of inflammation remained actively inflamed for a minimum of four weeks despite aggressive and appropriate medical or surgical therapy (Fig. 4).

The subjective vision status prior to, and following, IVGI was graded as *"**good**"* (positive menace, dazzle, direct and indirect PLR, with no evidence of obvious visual field impairment due to corneal edema, hyphema, hypopyon, fibrin in the anterior chamber, miosis, synechia, lens opacities/cataracts, vitreal degeneration, fundus abnormalities), *"**reduced**"* (positive menace, dazzle, direct and indirect PLR with some evidence of visual field impairment due to the abnormalities listed above) and *"**poor**"* (negative or positive menace, dazzle and direct and indirect PLR with obvious evidence of visual field impairment (late immature to mature cataracts), retinal degeneration/detachment, or phthisis bulbi).

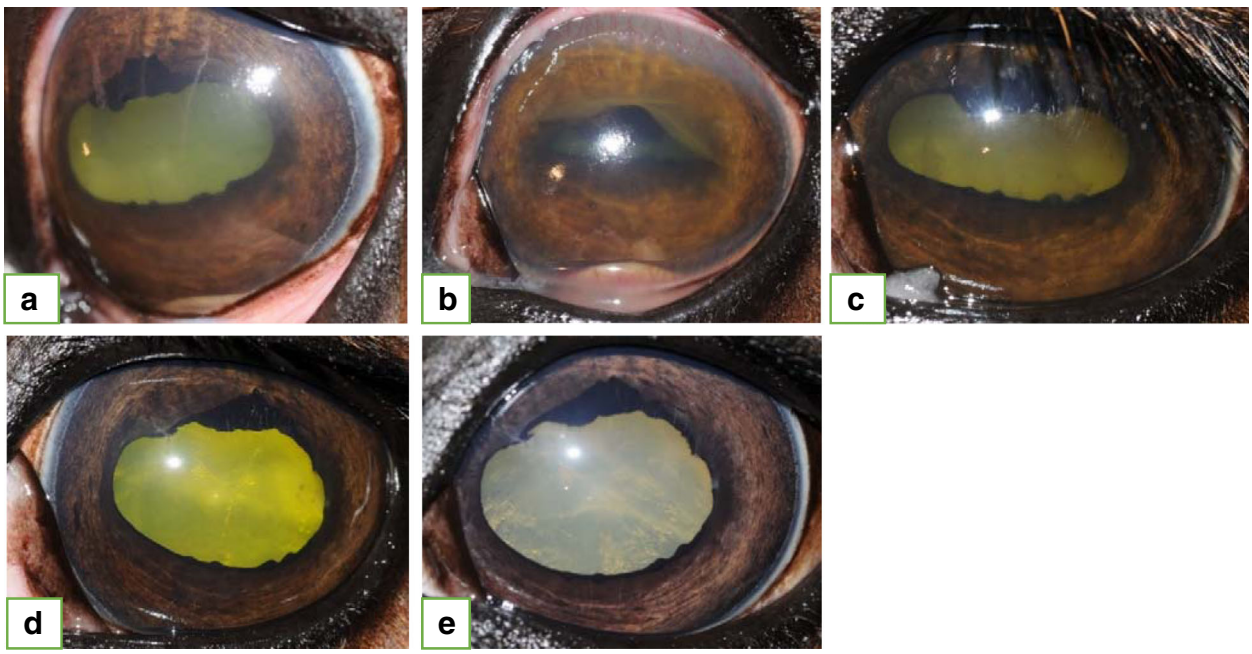

Fig. 4 Twelve-year old warmblood mare that presented for a chronic-acute persistent panuveitis of the left eye (OS). Negative c-value for *Leptospira*. **a.** Initial presentation: Diffuse corneal edema, keratic precipitates, + 2/4 flare, fibrin, vitreal degeneration and retinal folds were present. Medical treatment was started (prednisolone acetate q 4–6 h, atropine q 8 h and flunixine meglumine 1.1 mg/kg twice daily). **b.** Ten days post initial presentation: The clinical signs worsened in the face of aggressive medical treatment. Intravitreal gentamicin injection and aqueocentesis were performed OS. **c.** Four days post-IVGI OS: Improvement of clinical signs can be readily appreciated. **d.** Twenty days post-IVGI OS: No flare present. **e.** Forty-nine days post-IVGI OS: Uveitis remains controlled without medications. The mare was euthanized due to complications associated with cervical spinal fracture 2 months after IVGI

Sedation, intravitreal gentamicin injection and aqueous paracentesis

A general physical examination including auscultation and body temperature measurement was performed prior to sedation with a combination of detomidine hydrochloride (Domosedan, 0.01–0.02 mg/kg bwt i.v.)[8] and butorphanol (Alvegesic, 0.005–0.01 mg/kg bwt i.v.)[9] intravenous and intramuscular bolus (0.02–0.04 mg/kg bwt i.m.) injections. Blood (serum and EDTA) was drawn and submitted for a complete blood count and chemistry panel. A microscopic agglutination test (MAT) for *Leptospira* antibodies was also routinely performed [32]. A total of nine serovars (*L. bratislava,*[10] *L. icterohaemorrhagiae/copenhageni*[10], *L. australis*[10], *L. pomona*[10], *L. grippotyphosa*[10], *L. autumnalis*[10], *L.canicola,*[11] *L. saxkoebing*[11], *L.sejroe*[11]) were evaluated[10,11] .

Following sedation, the horse's head was positioned atop a pair of pads placed on a mobile cart to increase stability. Local akinesia and analgesia (palpebral and the frontal nerve blocks) was achieved using 2% mepivacaine (Scandicain 2%)[12] [33]. Topical anesthetic ophthalmic solution (proparacaine HCL 0.5%)[13] was applied as needed. The conjunctival fornices were irrigated with 1.0 ml of a dilute baby shampoo solution[14] (1 ml of baby shampoo in 1 l of balanced saline solution), 1.0 ml of a 1.0% dilute iodine solution,[15] and 1.0 ml of balanced saline solution (Acrisol)[4] [34].

Dorsal globe exposure was facilitated with either a Desmarres[16] or prototype eyelid retractor,[17] and further enhanced by rotating the horse's head away from the examiner to exaggerate ventral globe rotation. The first 34 horses were treated with a 4 mg injectable gentamicin solution containing preservatives (Genta100; 100 mg/ml)[9]. All additional horses ($n = 52$) were treated with preservative-free gentamicin (PFG) (Gentamicin-ratiopharm, 160 mg/2 ml SF).[18] Undiluted gentamicin (0.04 ml Genta100 or 0.05 ml PFG) [9,18] was drawn up in a 30- gauge needle/syringe combination (12 mm length, 1.0 ml insulin syringe), and the IVGI was performed using headloupes (magnification) (Eschenbach MaxView with LED light source or headloupes with a separate head-mounted light source (ML4-LED))[19,20,.21] The injection site was 10 mm posterior to the limbus at 12 o'clock. Injection was facilitated by applying gentle but steady pressure while slowly and deliberately rotating the needle in a clockwise manner with the needle directed toward the optic nerve head to avoid inadvertent contact with the lens. Aqueous paracentesis was then performed using a second insulin syringe at either the 11:00 o'clock (right eye, oculus dexter, OD) or 1:00 o'clock (left eye, oculus sinister, OS) positions. A total volume of 1 ml aqueous humor was aspirated. Aqueous humor and serum samples were refrigerated prior to transport to the laboratory. MAT tests for *Leptospira* titers were performed with the serum and aqueous humor, and real-time PCR was used for the detection of *Leptospira* DNA in the aqueous humor [35]. With the help of the C-value (dividing the aqueous humor *Leptospira* antibody titer by the serum *Leptospira* antibody titer), eyes were categorized into ***Leptospira* "positive"** (C-value greater than 3), ***"Leptospira* suspicious"** (C-value between 1 and 3) and ***"Leptospira* negative"** (C-value of 0) for statistical evaluation [27, 36].

Post-injection therapy

Post IVGI medical therapy consisted of topical antibiotics (Ofloxacin)[22] q8h for one week, and topical corticosteroids (Prednisolone acetate)[23] or nonsteroidal anti-inflammatory drugs (NSAIDs) (Bromfenac)[22] that were gradually tapered over the course of 4–8 weeks based on each horse's individual response to therapy. Either 1% tropicamide[24] or 1% atropine[25] were applied topically for a variable duration to maintain or achieve mydriasis and to stabilize the blood aqueous barrier. Systemic NSAIDs (flunixin-meglumine, 0.55 mg/kg, p.o., q12h)[9] were also administered per os and gradually tapered-off over the course of 7 to 14 days. A prophylactic dose of 37% omeprazole (Gastrogard, 2 mg/kg p.o.)[26] was routinely administered orally, once daily while using systemic NSAIDs.

Follow-up examination

Following IVGI all eyes were immediately examined for the presence of peri-injection complications (subconjunctival or intracameral hemorrhage), and re-examined within 24 h. Horses were monitored weekly for the first month or until medications were discontinued. Subsequent follow-up examinations were spaced further apart based on the horse's individual response to treatment. Inflammation was considered controlled if no signs of recurrent or persistent uveitis, independent of complications, were identified at any follow-up examination after medications had been discontinued. Particular attention was paid at all times to the possible development of post-injection complications (cataract formation/maturation, retinal degeneration).

Data analysis

All 86 eyes were evaluated for peri-injection complications (subconjunctival and intracameral hemorrhage), but only those with a minimum follow-up period of 30 days (59 eyes) were included in the post-injection statistical data evaluation. These latter eyes were monitored for signs of recurrent or persistent inflammation, as well as for the presence of additional complications or sequelae (e.g., cataract formation/progression and retinal degeneration). Correlation between the outcome (controlled, recurrent, or persistent inflammation), post-injection complications (no complications, cataract formation or maturation, and retinal degeneration), and possible influencing factors (breed, coat

color, gender, clinical diagnosis with chronic or acute and recurrent or persistent, Leptospira status of the eye, Leptospira PCR, individual C-values for each *Leptospira* serovar, the combined C-value, the *Leptospira* serum and aqueous humor titer, response to topical medication prior to IVGI, severity of signs, frequencies of recurrence, gentamicin with preservatives, PFG, visual reflex tests, vision status prior to IVGI, miosis before IVGI, subconjunctival hemorrhage, intracameral hemorrhage and glaucoma prior to IVGI) were determined using a Pearson's chi-squared test or a Fisher's exact test. To avoid same-animal correlation, a single eye per horse was randomly selected for inclusion in the study from those receiving bilateral IVGI. Differences with $P \leq 0.05$ were considered significant. Results were calculated using computerized statistical software (IBM SPSS 23.0).[27]

Endnotes

[1]Kowa Company Ltd., Tokyo, Japan
[2]Keeler Instruments Inc., Broomall, Pennsylvania, USA
[3]Icare, Oy, Vantaa, Finland
[4]Alcon, Pharma GmbH, Freiburg im Breisgau, Germany
[5]Nikon GmbH, Düsseldorf, Germany
[6]Life Pixel, Mukilteo, Washington, USA
[7]Kruuse A/S, Langeskov, Denmark
[8]Pfizer Animal Health, Exton, Pennsylvania, USA
[9]CP-Pharma, Handelsges. mbH, Burdorf Germany
[10]Idexx Laboratories, Inc., Ludwigsburg, Germany
[11]Laboklin GmbH und Co.KG, Bad Kissingen, Germany
[12]AstraZeneca GmbH, Wedel, Germany
[13]Ursapharm Azneimittel GmbH, Saarbrücken, Saarland
[14]Johnson and Johnson GmbH, Neuss Germany
[15]aniMedica GmbH, Senden-Bösensell, Germany
[16]B.Braun Welsungen AG, Welsungen, Germany
[17]Eickemeyer-Medizintechnik für Tierärzte KG, Tuttlingen, Germany
[18]Ratiopharm GmbH, Ulm, Germany
[19]Eschenbach Optik GmbH, Nürnberg, Germany
[20]Zeiss, Jena, Germany
[21]Heine Optotechnik, Herrsching, Germany
[22]Bausch and Lomb GmbH., Berlin, Germany
[23]Dr. Winzer GmbH, Berlin, Germany
[24]Pharma Stulln GmbH, Stulln, Germany
[25]Römhild pharmacy, Diessen, Germany
[26]Merial GmbH, Hallbergmoos, Germany
[27]IBM, Armonk, New York, USA

Abbreviations

AH: Aqueous humor; CSI: Cyclosporine implant; ERG: Electroretinogram; ERU: Equine recurrent uveitis; HIK: Heterochromic iridocyclitis and keratitis; IVGI: Intravitreal gentamicin injection; MAT: Microscopic agglutination test; NSAID: Nonsteroidal anti-inflammatory drug; OCT: Optical coherence tomography; PFG: Preservative-free gentamicin; PLR: Pupillary light reflex; PPV: Pars plana vitrectomy; S: Serum

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author (RJM) on reasonable request.

Authors' contributions

BMF: Data collection, manuscript preparation, revision and editing; RJM: Study design, data collection, manuscript preparation, revision and editing; SR: Statistical evaluation and manuscript revision; WB: Study design, manuscript preparation, revision and editing. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Owners were educated on the various medical and surgical (i.e., PPV, CSI, and intravitreal injections) treatment options, and risks associated with each option. Verbal client consent to perform the IVGI was obtained following an in-depth discussion of potential complications including failure of the selected treatment option to control the disease, resulting in persistent/ recurrent inflammation with progression of ocular signs, and potential cataract maturation or development and retinal degeneration or detachment.

Consent for publication

not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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References

1. Deeg CA, Thurau SR, Gerhards H, Ehrenhofer M, Wildner G, Kaspers B. Uveitis in horses induced by interphotoreceptor retinoid-binding protein is similar to the spontaneous disease. Eur J Immunol. 2002;32:2598–606.
2. Gilger BC, Deeg C. Equine recurrent uveitis. In: Gilger BC, editor. Equine ophthalmology, 2nd Edition. Missouri: Elsevier Science; 2010. p. 317–49.
3. Allbaugh RA. Equine recurrent uveitis: a review of clinical assessment and management. Equine Vet Educ. 2017;29:279–88.
4. Schwink KL. Equine Uveitis. Vet Clin North Am - Equine Pract. 1992;8: 557–74.
5. Miller TR, Whitley RD. Uveitis in horses. Mod Vet Pract. 1987;8:351–7.
6. Abrams KL, Brooks DE. Equine recurrent uveitis: current concepts in diagnosis and treatment. Equine Pract. 1990;12:27–35.
7. Rebhun WC. Diagnosis and treatment of equine uveitis. J Am Vet Med Assoc. 1979;175:803–8.
8. Gilger BC. Equine recurrent uveitis: the viewpoint from the USA. Equine Vet J. 2010;42(Suppl 37):57–61.
9. Werry H, Gerhards H. Zur operativen Therapie der equinen rezidivierenden Uveitis (ERU). Tierärztl Prax. 1992;20:178–86.
10. Frühauf B, Ohnesorge B, Deegen E, Boevé M. Surgical management of equine recurrent uveitis with single port pars plana vitrectomy. Vet Ophthalmol. 1998;1:137–51.

11. von Borstel M, von Oppen T, Glitz F, Frühauf B, Deegen E, Boevé MH, Ohnesorge B. Langzeitergebnisse der Pars plana Vitrektomie (double port) bei Equiner Rezidivierender Uveitis. Pferdeheilk. 2005;21:13–8.
12. Winterberg A, Gerhards H. Langzeitergebnisse der Pars-plana-Vitrektomie bei equiner rezidivierender Uveitis. Pferdeheilk. 1997;13:377–83.
13. Gilger BC, Wilkie DA, Clode AB, McMullen RJ, Utter ME, Komaromy AM, Brooks DE, Salmon JH. Long-term outcome after implantation of a suprachoroidal cyclosporine drug delivery device in horses with recurrent uveitis. Vet Ophthalmol. 2010;13:294–300.
14. Tömördy E, Hässig M, Spiess BM. The outcome of pars plana vitrectomy in horses with equine recurrent uveitis with regard to the presence or absence of intravitreal antibodies against various serovars of Leptospira interrogans. Pferdeheilk. 2010;26:251–4.
15. Gilger BC, Michau TM. Equine recurrent uveitis: new methods of management. Vet Clin North Am - Equine Pract. 2004;20:417–27.
16. Spiess BM. Equine recurrent uveitis: the European viewpoint. Equine Vet J. 2010;42(Suppl 37):50–6.
17. Gerding JC, Gilger BC. Prognosis and impact of equine recurrent uveitis. Equine Vet J. 2016;48:290–8.
18. Sandmeyer LS, Bauer BS, Feng CX. Equine recurrent uveitis in western Canada: a retrospective study. In: Dorothy Havemeyer equine ophthalmology symposium, Malahide, Ireland, June 2016. International equine ophthalmology consortium; 2016. p. 20–1.
19. Van KB, Rothova A, De VP. The pros and cons of intravitreal triamcinolone injections for uveitis and inflammatory cystoid macular edema. Ocul Immunol Inflamm. 2006;14:73–85.
20. Kok H, Lau C, Maycock N, McCluskey P, Lightman S. Outcome of intravitreal triamcinolone in uveitis. Ophthalmology. 2005;112:1916.e1–7.
21. Yi NY, Davis JL, Salmon JH, Gilger BC. Ocular distribution and toxicity of intravitreal injection of triamcinolone acetonide in normal equine eyes. Vet Ophthalmol. 2008;11(Suppl 1):15–9.
22. Baath J, Ells a L, Crichton a, Kherani a, Williams RG. Safety profile of intravitreal triamcinolone acetonide. J Ocul Pharmacol Ther. 2007;23: 304–10.
23. Douglas LC, Yi NY, Davis JL, Salmon JH, Gilger BC. Ocular toxicity and distribution of subconjunctival and intravitreal rapamycin in horses. J Vet Pharmacol Ther. 2008;31:511–6.
24. Wollanke B, Rohrbach BW, Gerhards H. Serum and vitreous humor antibody titers in and isolation of Leptospira interrogans from horses with recurrent uveitis. J Am Vet Med Assoc. 2001;219:795–800.
25. Pinard C. Gentamicin injection. In: 36th annual meeting of the American College of Veterinary Ophthalmologists, Nashville, Tennessee, October 2005. Vet Ophthalmol. 2005:437–50.
26. McMullen RJ, Fischer BM. Medical and Surgical Management of Equine Recurrent Uveitis. Vet Clin North Am-Equine Pract. 2017;33:465–81.
27. Goldmann HT, Witmer R. Antikörper im Kammerwasser. Ophthalmologica. 1954;127:323–30.
28. Romeike A, Brügmann M, Drommer W. Immunohistochemical studies in equine recurrent uveitis (ERU). Vet Pathol. 1998;35:515–26.
29. Kleinpeter A, Pohlmann A, Mütze M. Intravitreale Gentamicin-Injektion zur Therapie der equinen rezidivierenden Uveitis-Methode und Fallauswertung. Leipzig: Leipziger Tierärztekongress; 2014. p. 27–30.
30. Lam DL, Axtelle J, Rath S, Dyer A, Harrison B, Rogers C, Menon N, Van GRN. A Rayleigh scatter-based ocular flare analysis meter for flare photometry of the anterior chamber. Transl Vis Sci Technol. 2015;4:7.
31. Pinto NI, McMullen RJ, Linder KE, Cullen JM, Gilger BC. Clinical, histopathological and immunohistochemical characterization of a novel equine ocular disorder: Heterochromic iridocyclitis with secondary keratitis in adult horses. Vet Ophthalmol. 2015;18:443–56.
32. Brehm S, Weber A. Zum serologischen Nachweis von Leptospireninfektionen bei Rindern und Pferden mit Hilfe eines im Handel erhaltlichen Objektträgeragglutinationstestes. Der praktische Tierarzt. 1984; 65:835–41.
33. Gilger BC, Stoppini R. Equine ocular examination: routine and advanced diagnostic techniques. In: Gilger BC, editor. Equine ophthalmology, 2nd Edition. Missouri: Elsevier Science; 2011. p. 1–51.
34. Brooks DE, Matthews A, Clode AB. Disease of the cornea. In: Gilger BC, editor. Equine ophthalmology, 3rd Edition. Ames: John Wiley & Sons, Inc.; 2017. p. 252–368.
35. Stoddard RA, Gee JE, Wilkins PP, McCaustland K, Hoffmaster AR. Detection of pathogenic Leptospira spp. through TaqMan polymerase chain reaction targeting the LipL32 gene. Diagn Microbiol Infect Dis. 2009;64:247–55.
36. De Groot-Mijnes JD, Rothova A, van Loon AM, Schuller M, ten D-VLNH, de BJH, Schuurman R, Weersink AJL. Polymerase chain reaction and Goldmann-Witmer coefficient analysis are complimentary for the diagnosis of infectious uveitis. Am Journal Ophthalmol. 2006;141:313–8.

3.2 Medical and Surgical Management of Equine Recurrent Uveitis

Own contribution:

The following tasks which are part of the second publication, have been performed by myself

- Literature research
- Evaluation of own clinical experience
- Manuscript preparation

The tasks of the other author of this publication were the study design, manuscript preparation, revision and editing (Dr. R. McMullen).

Medical and Surgical Management of Equine Recurrent Uveitis

Richard Joseph McMullen Jr, Dr med vet[a,*],
Britta Maria Fischer, DVM[b]

KEYWORDS

• Equine recurrent uveitis • Cyclosporine implant • Vitrectomy • Intravitreal injection
• Gentamicin

KEY POINTS

- Primary uveitis (isolated bouts of inflammation) must be differentiated from recurrent uveitis (multiple bouts of inflammation interrupted by periods of quiescence).
- Medical therapy/management alone leads to severe loss of vision or blindness in greater than 50% of all affected horses.
- There is a breed predilection for ERU in Appaloosa, draft, Knabstrupper, Icelandic, and warmblood breeds.

INTRODUCTION

Equine recurrent uveitis (ERU) is a widely recognized, complicated, multifaceted disease that is characterized by multiple, recurrent bouts of inflammation interrupted by variable periods of quiescence.[1–6] True recurrences of inflammation occur following complete elimination of inflammatory signs (eg, keratic precipitates [KPs], aqueous flare, miosis, cortical [equatorial] cataracts, vitreal opacification, fundus or optic nerve head [ONH] lesions) via topical and systemic antiinflammatory and immunosuppressive medication.[1,5,6] When medical therapy is withdrawn too soon, it may appear as if the inflammation returns within a short period of time (often 2–6 weeks). However, in many cases the signs associated with ERU had not been completely eliminated, but merely suppressed, giving the appearance that the eye had reached a stage of

Disclosure: The authors have nothing to disclose.
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quiescence. This premature cessation of medications often occurs if the eyes become comfortable and subtle signs of inflammation (eg, KPs, aqueous flare, vitritis, inflammation of the ONH [optic neuritis]) are missed during reexamination (**Fig. 1**). This situation is referred to as a pseudorecurrence and can lead to a misdiagnosis or, worse, to progressive intraocular changes resulting in decreased vision or blindness if it goes undetected.[2]

A recent study from western Canada reported that 12 out of 32 (38%) horses with ERU were bilaterally blind on presentation and 20 out of 26 (76.9%) were bilaterally blind at the last follow-up, and 17 out of 20 (85%) of these blind horses were euthanized.[7] In another study from the southeastern United States, 96 out of 338 (28%) of the eyes presenting with ERU were blind on initial presentation.[8] Forty-one out of 338 (12.1%) eyes were enucleated and 29 out of 224 (14.9%) of the horses were euthanized.[8] Both of these studies reveal that too many horses are being referred far too late in the disease process (**Fig. 2**).[7,8] Therefore, it is essential that horses showing subtle clinical signs that are not immediately associated with ERU (intermittent redness [conjunctival hyperemia], tearing [epiphora], squinting [blepharospasm]) should be thoroughly examined for additional signs associated with chronic or recurrent uveitis (KPs, aqueous flare, miosis, decreased intraocular pressure [IOP]). This approach will allow for targeted therapy to be administered early in the disease process, which may prevent more severe secondary complications from developing, and will initiate a reevaluation pattern by owners, referring or primary veterinarians, and veterinary ophthalmologists alike, which may increase the likelihood of preserving vision.

There are several alternative treatment approaches that may prove useful in the earlier stages of intervention and may result in fewer horses losing vision or requiring more invasive surgical intervention to control inflammation caused by ERU. Such treatment options include intravitreal gentamicin (IVG) injections,[9–11] intravitreal triamcinolone injections,[12] intravitreal rapamycin injections,[13] suprachoroidal space injections of triamcinolone,[14] surgical placement of suprachoroidal cyclosporine sustained-release devices (cyclosporine implants),[15,16] and pars plana vitrectomy.[17–19] Diagnosing ERU and selecting the most appropriate treatment option is tedious, difficult, and riddled with setbacks. Conservative medical therapy provides the foundation of therapy and should be initiated in every case

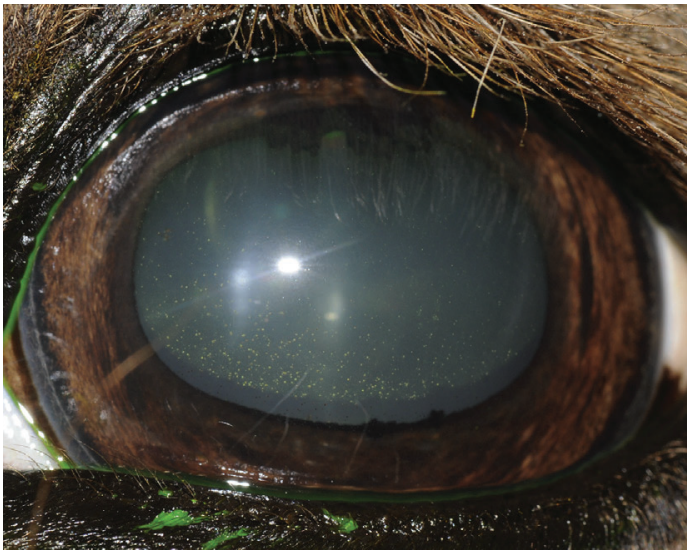

Fig. 1. Although the eye is open and comfortable, a moderate number of endothelial KPs remain visible during direct retroillumination. The dark, pinpoint KPs appear refractile during retroillumination. The pupil has been pharmacologically dilated.

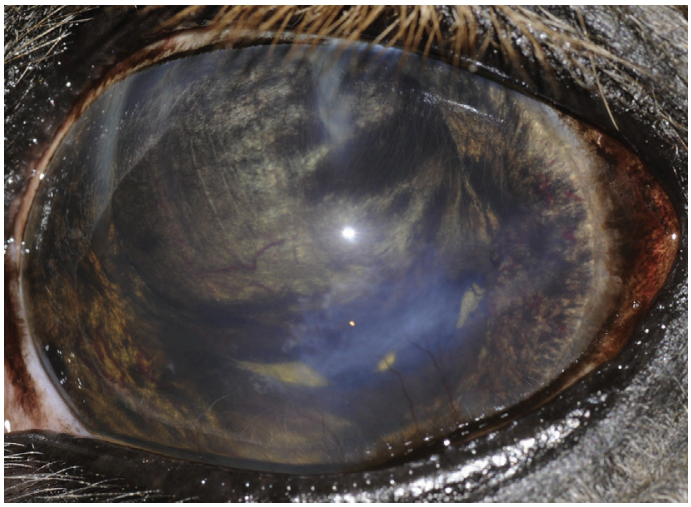

Fig. 2. Chronic and insidious panuveitis with corneal fibrosis and neovascularization, a shallow anterior chamber, rubeosis iridis (vascular engorgement of the iris vessels), and a ventrally displaced pupil (corectopia). The anteriorly displaced lens is entrapped posterior to the dorsal iris (note the spherical anterior protrusion in the dorsal portion of the iris).

of uveitis. Once all signs of inflammation have resolved, medical therapy can be discontinued. It is important that the antiinflammatory and immune-suppressive drugs are tapered off over a prolonged course of treatment (generally, 6–8 weeks) for 2 reasons. First, if the inflammation is not well controlled, clinical signs may worsen during the slow tapering of drugs, allowing for prompt increases in medication frequencies to again quickly suppress the inflammation. Second, stopping topical and systemic antiinflammatory and immunosuppressive therapy after 14 to 21 days, when subtle signs of inflammation (aqueous flare, pinpoint KPs) may be overlooked without careful examination, leads to the development of pseudorecurrences.[2] The return of bouts of inflammation in these situations is not caused by a new round of active uveitis but by a slow resurfacing of clinical signs of uveitis associated with the previous bout of insufficiently suppressed inflammation (**Fig. 3**).

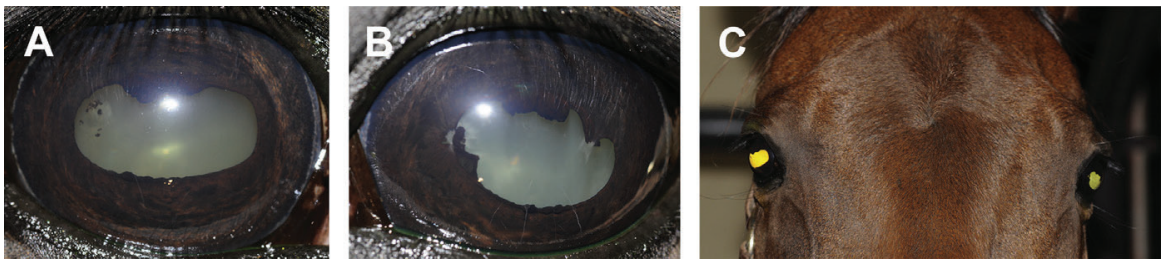

Fig. 3. (*A*) Right eye (oculus dexter [OD]) of a 20-year-old thoroughbred mare with chronic ERU 2 weeks following bilateral low-dose intravitreal injections of preservative-free gentamicin (4 mg) and topical and systemic antiinflammatory and immunosuppressive treatment. The pupil is only midrange, despite topical treatment with atropine. Note the degeneration of the corpora nigra and focal pigment deposition along the anterior lens capsule. Peripheral corneal neovascularization remains present, but is difficult to see because of the darkly pigmented iris. (*B*) Left eye (oculus sinister [OS]) of the horse from (*A*). The pupil is irregularly shaped (dyscoria), the corpora nigra show moderate degeneration, and there are focal adhesions between the pupil and anterior lens capsule (posterior synechiae) at both the 2:30 and 8:00 o'clock positions. Peripheral corneal neovascularization is present, but difficult to visualize, in this eye too. (*C*) A view of the same mare from the front reveals a yellow tapetal reflex, indicating that there is still significant inflammation present in both the anterior chamber and vitreous of both eyes (oculus uterque [OU]).

That is, these eyes never reach a true state of quiescence (periods without inflammation between separate bouts of active inflammation). Unrecognized pseudorecurrences may be mistaken for recurrent bouts of inflammation, suggesting that the underlying inflammation is more severe than may be the case. However, the development of pseudorecurrences can prove to be just as serious, if not more so, as a recurrent bout of inflammation recurring after a longer period of quiescence because the increased signs associated with the recurrences cause repeated and amplified damage to an already vulnerable eye.[2] Ensuring that a true state of quiescence is reached by gradually tapering medications over a prolonged period of time helps to accurately assess the horse's underlying state of inflammation. Recurrences that occur frequently (every 3–4 months, or less), and require longer durations of treatment before the signs of inflammation subside, are more likely to develop debilitating ocular complications associated with ERU (marked aqueous flare, fibrin accumulation in the anterior chamber [**Fig. 4**], posterior synechia [**Fig. 5**], vitritis, retinal folds or degeneration [**Fig. 6**], optic neuritis [**Fig. 7**]) or go blind than eyes with annual recurrent bouts of inflammation associated with minimal signs of disease (trace to mild flare, fine KPs [see **Fig. 1**], and miosis [**Fig. 8**]). Presently, there are several treatment options to reduce or prevent recurrent inflammation and that help to maintain vision in horses with ERU.[9–19]

PATIENT EVALUATION OVERVIEW

A detailed history is essential and can help to identify initial and mild recurrences, which often go unnoticed. Episodes of conjunctival hyperemia (redness), tearing (epiphora), and/or squinting (blepharospasm) that wax and wane are often reported by owners as being present before any so-called real eye problems develop. Many horses with ERU have 1 or several of the episodes described earlier during the years before clinical presentation. A heightened awareness or sensibility for the findings described earlier may lead to earlier recognition, earlier diagnosis, and ultimately to the earlier implementation of targeted treatment (**Table 1**).

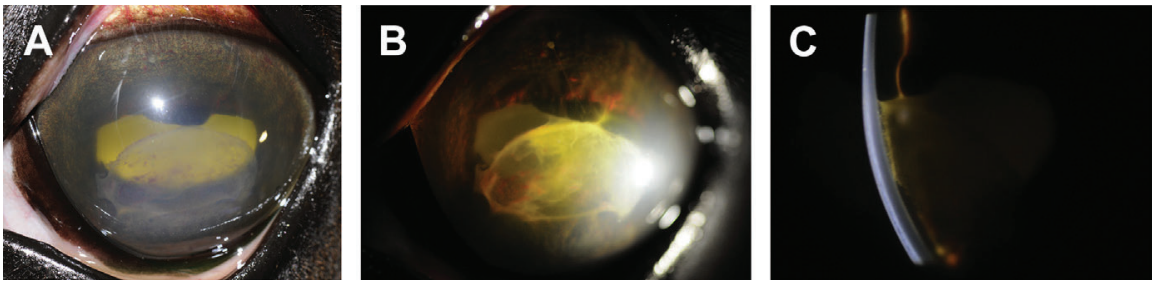

Fig. 4. (*A*) The left eye (OS) of a quarter horse yearling with a large fibrin clot in the anterior chamber. Note the other signs of severe uveitis: marked rubeosis irides, iris depigmentation and corpora nigra degeneration, and murky yellow tapetal reflex consistent with moderate aqueous flare and vitreal opacification. (*B*) Using tangential illumination (light directed obliquely from the temporal limbus) the superficial iris vessels (rubeosis irides) and the fibrin clot can be visualized with much more detail. Note the fibrin adhered to the corpora nigra along the dorsal edge of the pupil. (*C*) This handheld slit lamp image shows the thickened ventral cornea and partial adhesion of the fibrin to the corneal endothelium. Also note the smooth surface of the corpora nigra, which is visible just above the dorsal aspect of the fibrin clot occupying the entire depth of the anterior chamber (yellowish material between the white corneal light reflex and light brown slit of light along the surface of the iris).

Fig. 5. Chronic recurrent panuveitis with predominantly anterior signs. Note the small tuft of fibrin near the iridocorneal angle of the anterior chamber at 8:30 o'clock. There is also marked posterior synechiae and diffuse pigment deposition along the anterior lens capsule. Note the thin, white, membranous veil containing pigment (fine punctate spots) spanning the ventral width of the pupil.

- ERU may also be further differentiated according to stages of chronicity, with cases being labeled as active (**Fig. 9**), quiescent (**Fig. 10**), or end stage (**Fig. 11**).
- The following anatomic diagnoses may make differentiation easier: panuveitis; panuveitis with predominant posterior signs; panuveitis with predominant anterior signs; anterior uveitis; posterior uveitis (**Fig. 12**); and heterochromic iridocyclitis with secondary keratitis (**Fig. 13** and **Table 2**).[20]

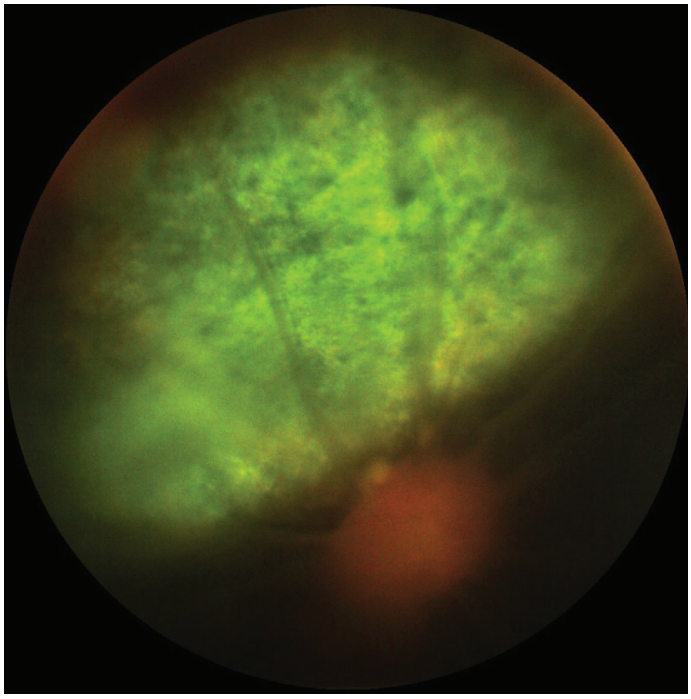

Fig. 6. The tapetal fundus of this 8-year-old quarter horse mare is markedly and diffusely hyperreflective, and there are multiple retinal folds manifest as linear bands extending radially from the ONH. This clinical presentation is very severe. Note the small area of subretinal cellular infiltrate along the 12:00 o'clock edge of the ONH.

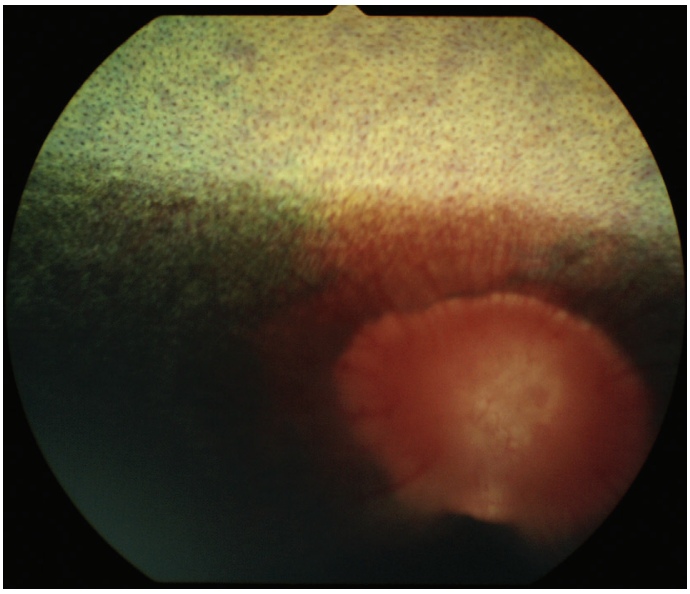

Fig. 7. The ONH in this horse with chronic recurrent panuveitis is moderately edematous and hyperemic. During binocular indirect ophthalmoscopy the anterior displacement of the thickened (edematous) peripapillary retina can be readily appreciated.

- With chronicity, regardless of which type of ERU is present, corneal vascularization, endothelial degeneration resulting in persistent corneal edema, linear corneal calcification (especially within and parallel to the palpebral margins), posterior (occasionally anterior) synechiae, cataract formation, and alterations in iris color and surface appearance commonly occur. Secondary glaucoma and phthisis bulbi can occur, ultimately resulting in irreversible blindness in many cases of ERU.

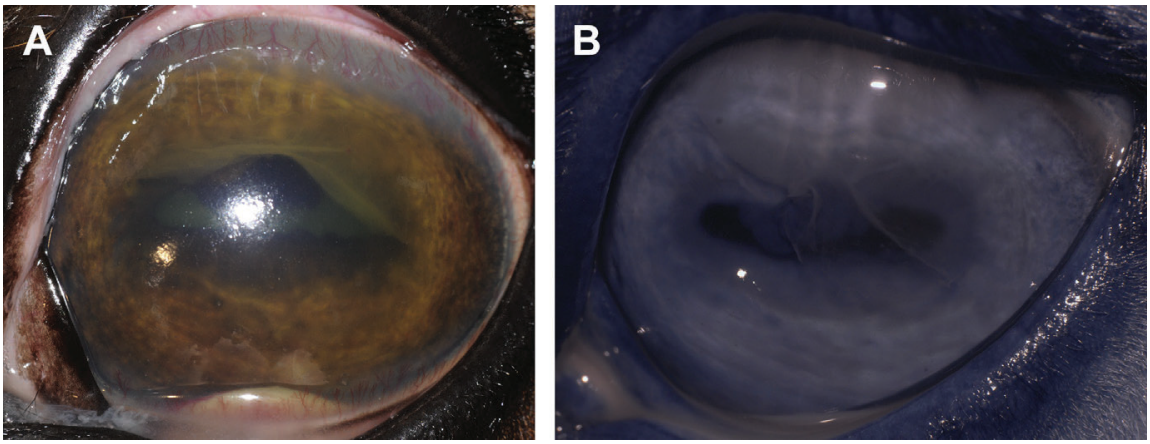

Fig. 8. (*A*) Miosis in the left eye (OS) of a 6-year-old quarter horse gelding. This active bout of inflammation recurred following early cessation of topical and systemic medications. This pseudorecurrence presented with marked blepharospasm, corneal neovascularization, and a shallow anterior chamber, with hypopyon (ventral iridocorneal angle), miosis, fibrin accumulation in the anterior chamber, and diffuse iris hypopigmentation. The corpora nigra are also atrophied (this is easier to visualize in the infrared image [*B*]). (*B*) Infrared image of OD from (*A*). Note that the pupil, degenerative/atrophied corpora nigra, and the fibrin veil in the anterior chamber are easier to visualize in the infrared image.

Table 1
Classification of equine recurrent uveitis

Categories of ERU	Description	Tissue Affected	Anatomic Diagnosis	Breed Predisposition
Classic	• Active bouts of inflammation • Followed by variable periods of quiescence	Primary: Uvea (iris, ciliary body, choroid) Secondary: Cornea, anterior chamber, lens, vitreous, retina	• Panuveitis • Panuveitis (anterior) • Panuveitis (posterior) • Anterior uveitis • HIK	Warmblood Icelandic horses
Insidious	• Low-grade intraocular inflammation • Not outwardly painful • Gradual tissue destruction • Degeneration of multiple intraocular structures	• Posterior segment inflammation initially • Anterior segment inflammation follows • End-stage eyes globally affected	• Panuveitis • Panuveitis (anterior) • Panuveitis (posterior) • HIK	Appaloosa Draft breeds Knabstrupper
Posterior	• Acute bouts of inflammation are severe and respond slowly to medical therapy	• Predominantly posterior segment inflammation • Mild anterior inflammation is common	• Posterior uveitis • Panuveitis (posterior) • Panuveitis	Warmblood

Abbreviation: HIK, heterochromic iridocyclitis with secondary keratitis.

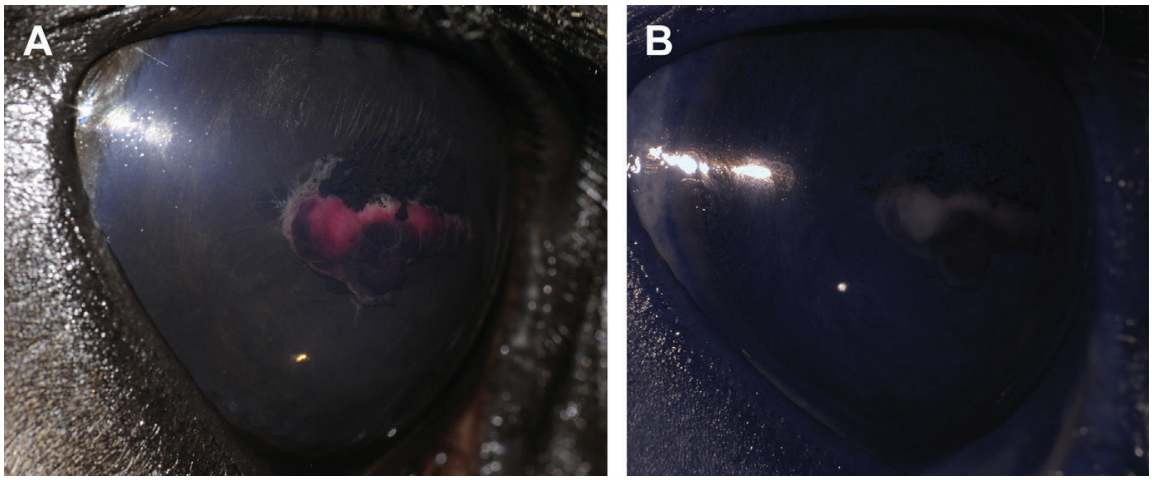

Fig. 9. (*A*) Active inflammation in the right eye (OD) of an 8-year-old bay warmblood mare with chronic recurrent panuveitis with predominantly anterior segment involvement. There is circumferential superficial corneal neovascularization and diffuse corneal haze, as well as a very shallow anterior chamber with complete miosis and fibrin and hyphema within the pupil. The iris is diffusely hyperpigmented and the posterior segment could not be visualized clinically. On ocular ultrasonography, only minimal vitreal hyperechogenicity could be appreciated. (*B*) Digital infrared image of the eye from (*A*). Despite the iris hyperpigmentation, a much better appreciation for the iridocorneal angle and pupil margin can be obtained with this method of clinical imaging.

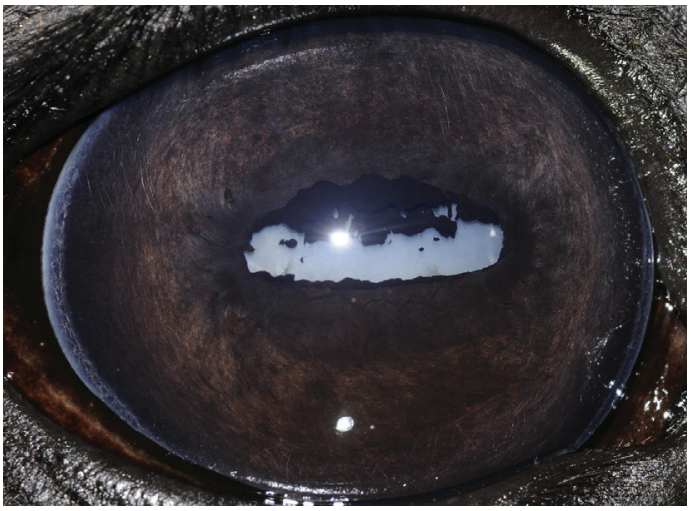

Fig. 10. Quiescent stage in the right eye (OD) from an 11-year-old bay warmblood gelding with a 3-year history of chronic recurrent inflammation that was only controlled while on high levels of immunosuppressive medications. He was ultimately treated with an intravitreal injection of low-dose gentamicin (4 mg). Although his eye had significant chronic signs of inflammation and secondary complications from ERU (he had significant discomfort, marked corneal vascularization, diffuse corneal fibrosis, complete miosis, and a mature cataract at the time of injection), he has remained free from recurrent bouts of inflammation for more than 602 days postinjection.

- It is important to consider/remember that current classifications of equine uveitis and ERU do not specifically differentiate between anatomic location (eg, anterior uveitis, posterior uveitis, and panuveitis) or clinical manifestation, but combine several potentially different clinical presentations into broader disease syndromes that are essentially a combination of several different individual classifications.

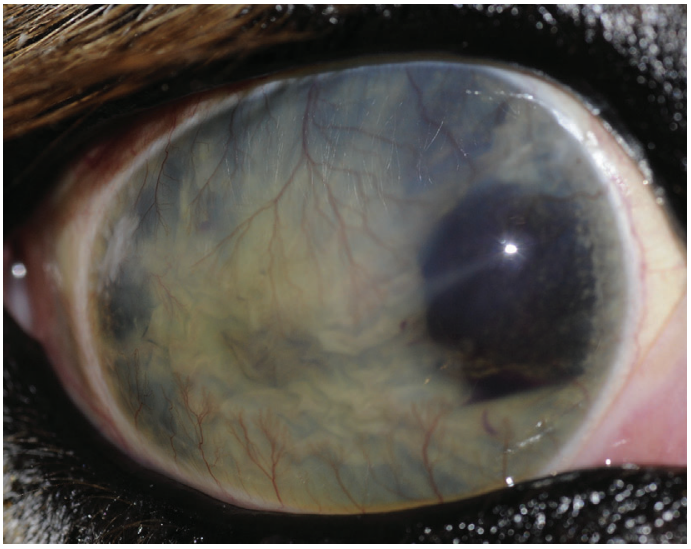

Fig. 11. End-stage uveitis. Phthisis bulbi (shrunken globe) with marked corneal vascularization, linear corneal fibrosis (representing folds in the cornea as a consequence of extremely low intraocular pressure), complete loss of anterior chamber depth, secluded pupil, and marked depigmentation of the iris. The posterior segment was not visible clinically, but ocular ultrasonography revealed moderate vitreal opacification and retinal detachment.

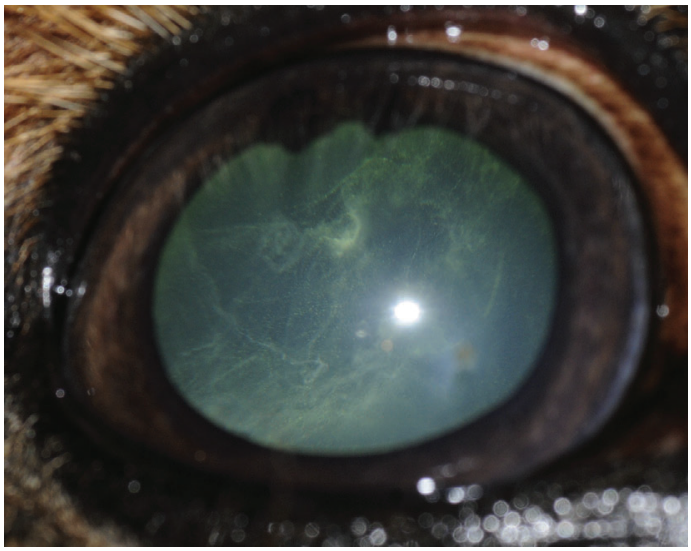

Fig. 12. Chronic, recurrent posterior uveitis in a 9-year-old Icelandic horse gelding. There is significant vitreal inflammation (vitritis) present, which can be readily identified through the pharmacologically dilated pupil. There are small, focal, anterior cortical cataracts associated with focal pigment deposition from recurrent bouts of inflammation but a relative lack of anterior segment signs.

PHARMACOLOGIC TREATMENT OPTIONS

Conventional Medical Therapy

Details of conventional medical therapy are given in **Tables 3–5**.

Long-term Control and/or Prevention of Recurrent Bouts of Inflammation

Conventional antiinflammatory and immunosuppressive medical therapy is necessary to reduce/eliminate inflammation and indirectly minimizes secondary ocular damage occurring as a result of each recurrent bout of inflammation. However, even when effective, conventional medical therapy cannot prevent recurrent bouts of inflammation. There are several other treatment options available that may effectively postpone or prevent such recurrences.

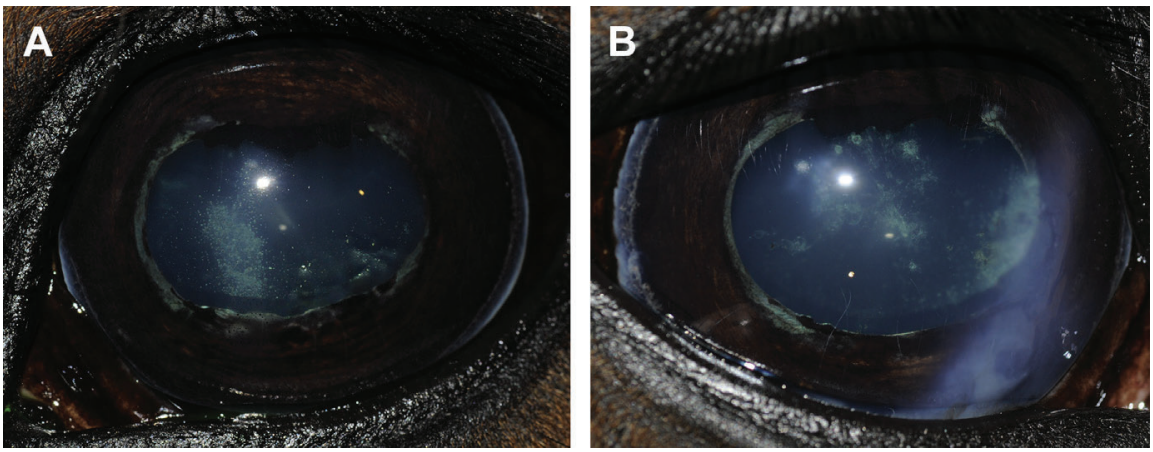

Fig. 13. (*A*) Left eye (OS) and (*B*) right eye (OD). Heterochromic iridocyclitis with secondary keratitis (HIK; endotheliitis) in a 12-year-old, bay warmblood gelding. Note the fine punctate (OS) and larger focal (OD) corneal opacifications and ventromedial areas of focal corneal edema. These findings, along with the diffuse depigmentation and iris atrophy/degeneration, are characteristic of this type of anterior uveitis.

Table 2
Clinical signs associated with equine recurrent uveitis

Categories of ERU	Clinical Signs		
	Acute	Chronic	Sequelae
Classic	• Increased lacrimation • Blepharospasm • Miosis • Photophobia • Aqueous flare • Intraocular fibrin • Hyphema • Hypopyon	• Miosis • Low IOP • Cataract formation/ progression • Phthisis bulbi	• Posterior synechiae • Misshapen pupil • Intermittent increases in IOP with chronicity • Severe vision loss and/ or blindness
Insidious	• Not outwardly painful ◦ Generally not detected until later stages of disease • Conjunctival and episcleral vascular hyperemia • Mild to moderate blepharitis • Aqueous flare	• Focal or diffuse corneal edema (dull or lackluster appearance) • Iris atrophy/corpora nigra degeneration • Iris discoloration ◦ Hyperpigmentation ◦ Hypopigmentation • Lens subluxation or luxation ◦ Anterior or posterior • Shallow anterior chamber	• Absent or sluggish pupillary light reflexes • Posterior synechiae • Pigment on anterior lens capsule • Pupillary occlusion • Focal/diffuse cataracts obscure visualization of the posterior segment • IOP generally low ◦ <12 mm Hg • Secondary glaucoma is common ◦ Grave prognosis for maintaining vision
Posterior	• Vitritis • Chorioretinal scarring • Retinal degeneration • Optic neuritis • Subtle anterior segment signs: ◦ KPs ◦ Aqueous flare ◦ Miosis ◦ Blepharospasm	• Active/inactive chorioretinitis • Focal or diffuse retinal detachments ◦ Peripapillary retinal folds • Vitreous: cloudy/hazy appearance • ONH and surrounding retina may appear congested	• Bullet-hole lesions • Butterfly lesions ◦ Prevalence associated with ERU unknown: lesions not commonly seen with ERU

NONPHARMACOLOGIC TREATMENT OPTIONS

Leptospira Vaccination

Leptospirosis, the intraocular (vitreous) sequestration of leptospiral antibodies, or the organism itself has been associated with ERU for decades.[21–28] Despite this fact, there is little known on the pathophysiology of leptospiral-induced ERU. Because leptospiral detection (antigen or antibodies) is not routinely performed, the prevalence of leptospiral-induced ERU remains enigmatic. A study from Zurich, Switzerland, described a useful protocol for ERU patient selection to better determine which horses are the best candidates for vitrectomy surgery.[29] A recent study from Germany, evaluating the use of low-dose IVG (4 mg) in which each horse was evaluated for the presence of leptospiral and equine herpesvirus (EHV) DNA (aqueous humor) and leptospiral antibody titers (serum and aqueous humor), showed that the overall exposure to leptospiral organisms is high (63 out of 79 eyes; 79.75%; C-value, 0–3), but that the presence of a C-value greater than 3 (indicating intraocular antibody production)

Table 3
Topical medications for equine recurrent uveitis

Drug Class	Medications	Frequency	Pros	Cons
Corticosteroid	Prednisolone acetate 1%	q 4–6 h	Potent Excellent ocular penetration	Immunosuppressive, predisposes to secondary corneal fungal infections
	Dexamethasone 0.1%	q 4–6 h	Potent Excellent ocular penetration	Immunosuppressive, predisposes to secondary corneal fungal infections
NSAIDs	Flurbiprofen, diclofenac, suprofen, or bromfenac	q 8–24 h	Good additional antiinflammatory medication alone or in conjunction with corticosteroids May be used when a corneal ulcer is present	May not be as effective as corticosteroids in acute phase of disease
Mydriatic	Atropine HCl 1%	q 4–24 h	Decreases iris muscle spasm (cycloplegia), induces mydriasis, minimizes synechia formation, stabilizes blood-ocular barriers	May decrease gut motility Monitor for signs of colic Pupil remains dilated for up to 21 d in normal eyes

Abbreviation: q, every.

was seen in only 16 out of 79 eyes (20.25%) of the horses tested.[11] The roe of EHV in ERU remains to be determined, but preliminary evaluation of the data mentioned earlier indicate that EHV-2 or EHV-5 DNA can be simultaneously detected in severe cases of ERU.[11] This information may become useful in the future when trying to determine the significance of leptospiral titers, especially in the context of vaccination. In addition, leptospiral vaccination in dogs can result in seroconversion, leading to increased titers from serovars other than Pomona.[30] If this occurs in horses, it will

Table 4
Subconjunctival medications for equine recurrent uveitis

Drug Class	Medications	Frequency	Pros	Cons
Corticosteroids	Methylprednisolone acetate (40 mg)	q 1–3 wk	Duration of action, 7–10 d	Markedly increased risk of secondary infections (fungal or bacterial) Cannot be removed once administered
	Triamcinolone acetonide (1–4 mg)	q 1–3 wk	Duration of action, 7–10 d	Markedly increased risk of secondary infections (fungal or bacterial) Cannot be removed once administered

Table 5
Systemic medications for equine recurrent uveitis

Drug Class	Medications	Frequency	Pros	Cons
NSAIDs	Flunixin meglumine (0.25–1.1 mg/kg IV, PO)	q 12–24 h	Potent and effective: ophthalmic disease	Chronic use may lead to gastric and renal toxicity
	Phenylbutazone (2.2–4.4 mg/kg IV, PO)	q 12–24 h	Moderately potent: ophthalmic disease	Chronic use may lead to gastric and renal toxicity Less effective than flunixin meglumine
Corticosteroids	Dexamethasone (6–10 mg/500 kg, PO or 2.6–6 mg/500 kg, IM)	q 24 h	Potent antiinflammatory	Use with caution and monitor for laminitis
	Prednisolone (100–300 mg/500 kg, IM, PO)	q 24 h	Potent antiinflammatory	Use with caution and monitor for laminitis

Abbreviations: IM, intramuscular; IV, intravenous; NSAIDs, nonsteroidal antiinflammatory drugs; PO, by mouth.

confuse things further. Therefore, it will become even more important in the future to evaluate both serum and aqueous humor for leptospiral titers and DNA and to correlate these results with the disease history and clinical signs present on presentation.[11,29]

COMBINATION THERAPIES

Intravitreal injections are routinely used in human ophthalmology to manage various forms of uveitis, and several medications have recently been evaluated in horses.[9–13] The data pertaining to the use of IVG injections have been anecdotal, and long-term follow-up data are presently not available.[9–11] A recent retrospective case series evaluating the efficacy of the IVG injections for various types and stages of ERU has been conducted and has influenced the way equine uveitis cases are managed.[11] The authors use a standard diagnostic protocol consisting of a complete and thorough ophthalmic examination by a board-certified veterinary ophthalmologist, which includes examination with a slit lamp biomicroscope, indirect ophthalmoscopy, tonometry, fluorescein staining of the external ocular structures, and color and infrared digital images, at a minimum. Ocular ultrasonography and fundus photography are performed as deemed necessary. Following examination and establishing a clinical diagnosis, the affected horses are sedated, local eyelid blocks are performed, and IVG 4-mg injections and aqueocentesis are performed.[11] The number of recurrences post-IVG injection is less than 15%. Therefore, the number of horses requiring surgical intervention or intensive long-term medical management is low. The complication rates associated with the injections are also low, but do consist of mature cataract formation/progression (5 out of 59 eyes; 8.5%) and/or retinal degeneration (3 out of 5 eyes; 5.1%).[11] Additional research and more long-term follow-up from a large number of treated horses will help to determine the true prevalence.[11]

Because aqueous paracentesis and IVG injections are performed under sedation, all risks associated with general anesthesia can be avoided, unless a horse requires

additional treatment because it has not responded to the initial IVG injection, and surgical placement of a cyclosporine implant or pars plana vitrectomy are deemed necessary.[15–19] This logical step-by-step approach has reduced the number of horses requiring surgery, while simultaneously decreasing the number of recurrent bouts of inflammation in our study population.[11] Anecdotally, it is common to inject gentamicin and triamcinolone intravitreally as a combination, but we have refrained from doing so in order to gain an appreciation of the efficacy of gentamicin in controlling ERU and preventing recurrent bouts of inflammation, as well as to minimize/eliminate the complications that may occur when using intravitreal corticosteroids. Within our study population, the use of triamcinolone acetonide has not been deemed necessary (**Table 6**).[11]

Another promising technique is the injection of triamcinolone acetonide into the suprachoroidal space.[14] This technique requires specially machined microneedles to perform, and potentially eliminates the secondary complications (corneal ulceration, secondary infection, corneal mineralization [**Fig. 14**], and endophthalmitis) associated with intravitreal triamcinolone injections.[13]

SURGICAL TREATMENT OPTIONS

There are currently 2 surgical options to treat ERU: suprachoroidal placement of sustained-release cyclosporine devices and dual-port pars plana vitrectomy.[15–19] It is commonly inferred that ERU in Europe is different from ERU seen in the United States.[1–4] Although there are geographic and breed-related differences that are more pronounced on either side of the Atlantic Ocean, there are more similarities than is generally supposed.[31,32]

Table 6
Medications for intravitreal and suprachoroidal space injections for equine recurrent uveitis

Route of Administration	Medications	Frequency	Pros	Cons
Intravitreal injection	Gentamicin (4 mg, preservative free)	Once	Potential to interrupt and stop recurrent bouts of inflammation	Mechanism of action unknown May cause cataract formation/ maturation or retinal degeneration
	Triamcinolone acetonide (2.5–5.0 mg)	As necessary based on clinical response	Duration of action, 4–9 mo (monitor intravitreal crystals)	Markedly increased risk of secondary infections (fungal or bacterial) and corneal degeneration Cannot be removed once administered
Suprachoroidal space injection	Triamcinolone acetonide (5.0 mg)	As necessary based on clinical response	Corneal drug concentration eliminated: drastically reduced risk of secondary infection	Special needles required (not commercially available)

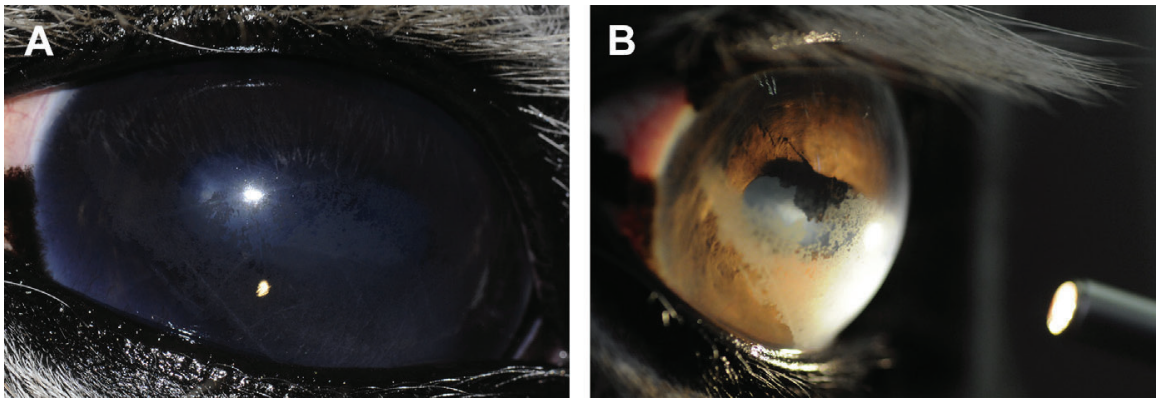

Fig. 14. (*A*) Diffuse corneal mineralization associated with intravitreal triamcinolone acetonide injection. This type of corneal mineralization is often anecdotally associated with the chronic use of topical corticosteroids (especially dexamethasone) as well. (*B*) Using oblique (or tangential) lighting as the sole source of illumination, the corneal mineralization of the cornea from (*A*) can be highlighted for better visualization.

Some of the misconceptions about ERU are linked to the simultaneous development of the pars plana vitrectomy[17–19] and intravitreal and subsequently suprachoroidal cyclosporine implants[15,33,34] in Germany and North Carolina, respectively. There is a greater population of Appaloosa and western sport horses in the United States compared with Germany's more dominant warmblood population. Coupled with the different examination techniques routinely used in each country, this accounts for some of the misconceptions. The many types and stages of ERU can be seen in both Europe and the United States, just at variable frequencies.

Suprachoroidal Cyclosporine Implants

Horses with documented recurrent bouts of inflammation that are well controlled with conventional medical therapy (eg, topical and systemic antiinflammatory medication effectively leading to a period of quiescence that remains even after medications are discontinued) are excellent candidates for suprachoroidal cyclosporine implantation.[33,34]

Inflammation can be well controlled and recurrences all but eliminated following placement of suprachoroidal cyclosporine implants.[34]

Pars Plana Vitrectomy

The pars plana vitrectomy, both single port and dual port, has been well described, and has seen widespread use, especially in Europe.[17–19,29,35] It is an intraocular procedure that is used primarily to remove the core of the vitreous with the horse under general anesthesia. The procedure is not routinely performed under an operating microscope and direct visualization of the vitrector (cutting instrument) is achieved using an indirect ophthalmoscope. Without the use of a condensing lens there is limited depth perception and extreme care must be taken not to inadvertently damage the posterior lens or retina. Although the procedure has seen widespread use in Europe, there are few studies evaluating the long-term surgical results following vitrectomy.[19,29,36] Reported postsurgical complications include transient hypopyon, vitreal and/or retinal hemorrhage, retinal detachment, and cataract formation.[1,19] Horses with *Leptospira*-associated uveitis (C-value >4), and moderate to severe vitreal inflammation (membranes) are considered good surgical candidates.[29]

EVALUATION OF OUTCOME AND LONG-TERM RECOMMENDATIONS

Conservative medical management is, and should be, the first line of treatment to stop active uveitis and to slow down or prevent recurrent bouts of inflammation. Once a horse has been diagnosed with ERU, it requires diligent and lifelong monitoring and care and the owners should be prepared for this.[8] If recurrences occur despite appropriate medical therapy,[6–8] then additional treatment modalities should be considered.

In these situations, intravitreal and suprachoroidal injections provide an effective alternative to surgery, and can be performed under sedation, at the same time as diagnostic aqueous paracentesis.[11–14,29] If the horse does not respond favorably to conservative medical or injection therapy, the best surgical option for each individual horse can be made based on the clinical signs that are still present, the anatomic diagnosis, and the laboratory results.[1,11,29,35]

SUMMARY

ERU is a complex and challenging disease, but clinicians are currently in a position to improve the long-term results with early intervention, making a proper diagnosis, and selecting the most appropriate treatment option for the individual horse. The reality is that more horses will go blind or experience debilitating ocular damage long term with medical therapy alone than with any other form of intervention.[1,7,8] Although the risks associated with the various injections and surgical options (cataract formation, retinal degeneration, retinal detachment, intraocular hemorrhage) remain present, the likelihood of their occurring can be dramatically reduced.[10,11,16,19,29,36]

REFERENCES

1. Gilger BC, Hollingsworth SR. Diseases of the uvea, uveitis, and recurrent uveitis. In: Gilger BC, editor. Equine ophthalmology. 3rd edition. Ames (IA): John Wiley; 2017. p. 369–415.
2. Lowe RC. Equine uveitis: A UK perspective. Equine Vet J 2010;(Suppl 37):46–9.
3. Spiess BM. Equine recurrent uveitis: the European viewpoint. Equine Vet J 2010;(Suppl 37):50–6.
4. Gilger BC. Equine recurrent uveitis: the viewpoint from the USA. Equine Vet J 2010;(Suppl 37):57–61.
5. Gilger BC, Michau TM. Equine recurrent uveitis: new methods of management. Vet Clin North Am Equine Pract 2004;20:417–27.
6. Allbaugh RA. Equine recurrent uveitis: a review of clinical assessment and management. Equine Vet Educ 2016. http://dx.doi.org/10.1111/eve.12548.
7. Sandmeyer LS, Bauer BS, Feng CX, et al. Equine recurrent uveitis in western Canada: a retrospective study. Dorothy Havemeyer Equine Ophthalmology Symposium. Malahide (Ireland). June 2–4, 2016. p. 20.
8. Gerding JC, Gilger BC. Prognosis and impact of equine recurrent uveitis. Equine Vet J 2016;48:290–8.
9. Pinard C. Gentamicin injection. American College of Veterinary Ophthalmologists Annual Conference. Nashville (TN). October 12–15, 2005.
10. Kleinpeter A. Intravitreale Gentamicin-Injektion zur Therapie der equinen rezidivierender Uveitis – Methode und Fallauswertung. Leipzig (Germany): Leipziger Tierärztekongress; 2014. 7.
11. Fischer BM, Brehm W, McMullen Jr RJ. Treatment of recurrent uveitis in horses with intravitreal low-dose gentamicin injection. Dorothy Havemeyer Equine Ophthalmology Symposium. Malahide (Ireland). June 2–4, 2016. p. 22.

12. Yi NY, Davis JL, Salmon JH, et al. Ocular distribution and toxicity of intravitreal injection of triamcinolone acetonide in normal equine eyes. Vet Ophthalmol 2008; 11(Suppl 1):15–9.
13. Douglas LC, Yi NY, Davis JL, et al. Ocular toxicity and distribution of subconjunctival and intravitreal rapamycin in horses. J Vet Pharmacol Ther 2008;31:511–6.
14. Gilger BC. Use of suprachoroidal injection of triamcinolone acetonide for treatment of non-responsive active uveitis. Dorothy Havemeyer Equine Ophthalmology Symposium. Malahide (Ireland). June 2–4, 2016. p. 24.
15. Gilger BC, Salmon JH, Wilkie DA, et al. A novel bioerodible deep scleral lamellar cyclosporine implant for uveitis. Invest Ophthalmol Vis Sci 2006;47:2596–605.
16. Gilger BC, Wilkie DA, Clode AB, et al. Long-term outcome after implantation of a suprachoroidal cyclosporine drug delivery device in horses with recurrent uveitis. Vet Ophthalmol 2010;13:294–300.
17. Werry H, Gerhards H. Möglichkeiten und Indikationen zur chirurgischen Behandlung der equinen rezidivierender Uveitis (ERU). Pferdeheilk 1991;7:321–31.
18. Werry H, Gerhards H. The surgical therapy of equine recurrent uveitis. Tierärztl Prax 1992;20:178–86.
19. Fruhauf B, Ohnesorge B, Deegen E, et al. Surgical management of equine recurrent uveitis with single port pars plana vitrectomy. Vet Ophthalmol 1998;1:137–51.
20. Pinto NI, McMullen RJ Jr, Linder KE, et al. Clinical histopathological and immunohistochemical characterization of a novel equine disorder: heterochromic iridocyclitis with secondary keratitis in adult horses. Vet Ophthalmol 2014. http://dx.doi.org/10.1111/vop.12234.
21. Davidson MG, Nasisse MP, Roberts SM. Immunodiagnosis of leptospiral uveitis in two horses. Equine Vet J 1987;19:155–7.
22. Dwyer AE, Crockett RS, Kalsow CM. Association of leptospiral seroreactivity and breed with uveitis and blindness in horses: 372 cases (1986-1993). J Am Vet Med Assoc 1995;207:1327–31.
23. Brem S, Gerhards H, Wollanke B, et al. Demonstration of intraocular *Leptospira* in 4 horses suffering from equine recurrent uveitis (ERU). Berl Münch Tierärztl Wochenschr 1998;111:415–7.
24. Brem S, Gerhards H, Wollanke B, et al. Leptospira isolated from the vitreous body of 32 horses with recurrent uveitis (ERU). Berl Münch Tierärztl Wochenschr 1999; 112:390–3.
25. Wollanke B, Rohrbach BW, Gerhards H. Serum and vitreous humor antibody titers in and isolation of *Leptospira interrogans* from horses with recurrent uveitis. J Am Vet Med Assoc 2001;219:795–800.
26. Faber NA, Crawford M, LeFebvre RB, et al. Detection of *Leptospira* spp. in the aqueous humor of horses with naturally acquired recurrent uveitis. J Clin Microbiol 2000;38:2731–3.
27. Halliwell RE, Brim TA, Hines MT, et al. Studies on equine recurrent uveitis. II: The role of infection with *Leptospira interrogans* serovar Pomona. Curr Eye Res 1985; 4:1033–40.
28. Gilger BC, Salmon JH, Yi NY, et al. Role of bacteria in the pathogenesis of recurrent uveitis in horses from the southeastern United States. Am J Vet Res 2008;69: 1329–35.
29. Tömördy E, Hässig M, Spiess BM. The outcome of pars plana vitrectomy in horses with equine recurrent uveitis with regard to the presence or absence of intravitreal antibodies against various serovars of *Leptospira interrogans*. Pferdeheilk 2010;26:251–4.

30. Barr SC, McDonough PL, Scipioni-Ball RL, et al. Serologic responses of dogs given a commercial vaccine against *Leptospira interrogans* serovar Pomona and *Leptospira kirschneri* serovar Grippotyphosa. Am J Vet Res 2005;66:1780–4.
31. Fritz KL, Kaese HJ, Valberg SJ, et al. Genetic risk factors for insidious equine recurrent uveitis in Appaloosa horses. Anim Genet 2014;45:392–9.
32. Kulbrock M, Lehner S, Metzger J, et al. A genome-wide association study identifies risk loci to equine recurrent uveitis in German warmblood horses. PLoS One 2013;8:e71619.
33. Gilger BC, Malok E, Stewart T, et al. Effect of an intravitreal cyclosporine implant on experimental uveitis in horses. Vet Immunol Immunopathol 2000;76:239–55.
34. Gilger BC, Wilkie DA, Davidson MG, et al. Use of an intravitreal sustained-release cyclosporine delivery device for treatment of equine recurrent uveitis. Am J Vet Res 2001;62:1892–6.
35. Dorrego-Keiter E, Tóth J, Dikker L, et al. Detection of *Leptospira* by culture of vitreous humor and detection of antibodies against *Leptospira* in vitreous humor and serum of 225 horses with equine recurrent uveitis. Berl Münch Tierärztl Wochenschr 2016;129:209–15.
36. Winterberg A, Gerhards H. Long-term results of pars plana vitrectomy in equine recurrent uveitis. Pferdeheilk 1997;4:377–83.

4 DISCUSSION

Useful diagnostic information pertaining to the ocular *Leptospira* status can be obtained by performing aqueous paracentesis at the time of IVGI. The information obtained is valuable, especially in cases where the inflammation cannot be controlled by IVGI. In such instances the indication for PPV or CSI can be readily made (TÖMÖRDY et al. 2010). When assessing the *Leptospira* status of the eye, it is imperative to compare the aqueous humor antibody titers to the serum antibody titers to determine if there is active intraocular antibody production (GOLDMANN and WITMER 2010,GILGER 2010). Failing to evaluate both the aqueous humor and serum antibody titers when evaluating the ocular *Leptospira* status, can be very misleading, as previous studies have shown that the serum antibody titer and the aqueous humor antibody titer are not correlated (FABER et al. 2000, MALALANA et al. 2017). Using PCR as the sole method to assess the *Leptospira* status of the eye, may also lead to misinformation, as nearly half of the uveitis cases associated with Leptospira would have been missed by relying only on PCR in one study (DE GROOT-MIJNES et al. 2006).

It is challenging to accurately categorize clinical uveitis cases by using the three historical categorizations classic ERU, insidious ERU and posterior ERU (GILGER and MICHAU 2004). There is definitely a need for a more modern and accurate categorization. Using the categorization of panuveitis, panuveitis with predominant anterior involvement, panuveitis with predominant posterior involvement, anterior uveitis, posterior uveitis and heterochromic iridocyclitis was an attempt to find a more precise way to categorize the different clinical presentations of uveitis (FISCHER et al. 2019). As a variety of clinical signs and even the recurrent nature of the disease, can also be found in other ocular diseases, such as IMMK, it is challenging to accurately make a correct diagnosis (REBHUN 1979, GILGER 2010). Future research should concentrate on categorizing uveitis based on clinical signs associated with uveitis and correlating them with clinical manifestations of the disease in order to identify reliable patterns of signs, associated with different clinical manifestations of uveitis.

The success rate of IVGI in our study was 88.1%, with vision threatening post-injection complications being identified in 8.5% (cataract maturation/formation) and 5.1% (retinal degeneration) of the cases, suggesting that IVGI may be a reliable treatment

option for horses presenting with various types and stages of uveitis. An additional benefit of IVGI is that it can be performed under sedation using minimal regional and topical anesthesia at the same time that aqueous humor is collected for diagnostic purposes (FISCHER et al. 2019). As a result, all risks associated with general anesthesia are consistently avoided. Being able to provide a minimally invasive method that can be performed under sedation may also be useful in non-visual and painful eyes, where owners are more reluctant to invest time, effort, and money towards a more expensive surgical procedure (PPV, CSI or even enucleation). Phthisical globes that are still actively inflamed may also benefit from IVGI (DEEG et al. 2007).

Presently, the mechanism of action of gentamicin on the disease process in ERU and other types of equine uveitis remains enigmatic. We can only speculate as to how gentamicin influences this autoimmune process. Positive suppression of inflammation in a variety of types and stages of equine uveitis was achieved following IVGI in the present study, despite the *Leptospira* status of the eye. A recent experimental study discovered that suppression of dihydroorotate dehydrogenase (DHODH), which plays a key role in the activation of lymphocytes, is capable of almost complete inhibition of experimentally-induced uveitis in rats (DIEDRICHS-MOHRING et al. 2015). Targeted suppression of de novo pyrimidine synthesis, requiring DHODH for the activation of lymphocytes is achieved by feeding PP-001, a third- generation molecule to rats with uveitis. Although purely speculative, the underlying mechanism of gentamicin may specifically, or unspecifically, block or suppress the activation of specific T-cell lines; cells that are known to play a significant role in autoimmune uveitis (ROMEIKE et al. 1998). Future research to uncover the ability of gentamicin to disrupt the inflammatory process is warranted and necessary.

Eyes with ERU from Appaloosas were temporarily controlled, but they all eventually went on to develop recurrent inflammation. Because ERU in Appaloosas tends to respond very differently than that seen in most other horses breeds to a variety of treatments (both medical and surgical), they should be monitored very closely for recurrences or relapses, despite appearing “normal” to their owners. Repeating IVGI in cases that do not respond to the initial IVGI may be an option, however, the cumulative effects of intraocular gentamicin will need to be determined prior to making any such recommendations. Additionally, 2/3 eyes from Appaloosas in the present

study were also diagnosed with IMMK in addition to uveitis. In general, most horses in the present study demonstrating unspecific recurrent ocular inflammation following IVGI presented with clinical manifestations of subclinical keratitis or secondary glaucoma, rather than recurrent uveitis. Therefore, it is important to carefully evaluate each horse presenting with suspected ‘recurrent uveitis’ for signs of these underlying diseases to ensure that a correct diagnosis is made, thus increasing the likelihood of a positive long-term outcome.

Eyes presenting with glaucoma concomitant to uveitis remained free of long-term-complications (cataracts or retinal degeneration). And, with the exception of one eye, all had positive clinical outcomes in terms of the uveitis remaining controlled. Nevertheless, glaucoma was not resolved following IVG. It has been previously reported that eyes with concomitant glaucoma and uveitis are more likely to be enucleated (GERDING and GILGER 2016). Presently, due to the poor short-term prognosis associated with these cases, several owners of horses affected with both glaucoma and uveitis ultimately elected to have these eyes enucleated. Since IVG offers an effective and minimally invasive means of controlling both acute and chronic uveitis, it’s role in the management of horses suffering from concomitant uveitis and secondary glaucoma should continue to be investigated.

The present study reports a positive outcome in 88.1% (non-recurrence/ non-persistence rate) of the eyes treated. These results substantiate the anecdotal findings reported by PINARD (2005) with a positive outcome of 94.4%, and those reported by KLEINPETER et al. (2019) showing a positive outcome in 91.8%.

Suprachoroidal triamcinolone injections present a promising method, with minimal side effects, of temporarily controlling ERU (GILGER et al. 2013, GILGER 2016). Based on the duration of effect (3-4 weeks), suprachoroidal triamcinolone acetonide may be an effective means of controlling active uveitis prior to or following other medical or surgical interventions (GILGER 2016). Horses receiving suprachoroidal injections of TA should be monitored closely for recurrent signs of uveitis, especially during the time-frame when the effects of the drug are expected to wane. Currently, the micro needles necessary to perform suprachoroidal injections in horses are not commercially available (MCMULLEN et al.2017).

The main goals in treating ERU are the reduction of ocular inflammation, the reduction or elimination of pain or discomfort, and the preservation of vision (GERDING and GILGER 2016, ALLBAUGH 2017). Medical therapy alone leads to significant vision loss due to the lack of long-term control of inflammation. Every bout of inflammation leads to more damage to the ocular structures and will ultimately lead to blindness (GERDING et al.2016). The introduction of the surgical techniques, PPV ad SCI, have improved these results, but only in cases where the indication for surgery is correct (WINTERBERG et al.1997, GILGER et al. 2010, TÖMÖRDYet al. 2010). PPV is indicated in cases with positive *Leptospira* C-values, whereas CSI is only effective at suppressing inflammation in horses that respond to immunosuppressive treatment (TÖMÖRDYet al. 2010, GILGER et al. 2010). It is therefore essential to have an accurate diagnosis and prior response to therapy in order to select the appropriate treatment approach. According to a recent study by FISCHER et al. (2019), IVGI is able to control inflammation despite the *Leptospira* status of the eye and despite the response to immuosuppressive treatment. Compared with published results following cyclosporine implant placement (46% non-recurrence rate, 16% cataract progression/formation and 16% retinal degeneration) and pars plana vitrectomy (73.6% - 100% non-recurrence rate, 38.2% - 44.2% cataract progression/formation, and 9.3% retinal degeneration) the complication rates associated with IVG injections (88.1% non-recurrence rate/non-persistance rate, 8.5% cataract progression/maturation, and 5.1% retinal degeneration) are low (WERRY and GERHARDS 1954, WINTERBERG and GERHARDS 1997, FRÜHAUF et al. 1998, VON BORSTEL et al. 2005, TÖMÖRDY et al. 2010, GILGER et al. 2010). This makes IVGI an excellent treatment option.

5 ZUSAMMENFASSUNG

Britta Maria Fischer

Intravitreale Injektion von niedrig dosiertem Gentamicin: eine alternative Methode zur Behandlung der equinen rezidivierenden Uveitis

KLINIK FÜR PFERDE DER VETERINÄRMEDIZINISCHEN FAKULTÄT DER UNIVERSITÄT LEIPZIG

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(52 Seiten, 18 Abbildungen, 13 Tabellen, 110 Literaturangaben)

Schlüsselwörter: Equine recurrent uveitis - intravitreale Injektion- Gentamicin – Leptospiren - Pferde Augenheilkunde

Zielstellung: Ziel der Arbeit war es, die Technik der intravitrealen Gentamicin Injektion darzulegen, die Auswirkungen dieser auf die klinischen Symptome von Uveitiden, sowie die möglichen unmittelbaren Komplikationen (innerhalb von 24 Stunden) und längerfristigen Komplikationen (30 bis 780 Tage) die mit dieser Technik verbunden sein können, zu beschreiben. Zusätzlich wurde der okuläre und systemische Leptospiren- Status ermittelt und der Einfluss dieser auf das Behandlungsergebnis untersucht.

Material und Methode: 86 Pferde verschiedenen Alters, Rasse und Geschlechts, mit wiederkehrender oder persistierender Uveitis, wurden mit 4mg einer unverdünnten Gentamicininjektion (0,04 ml, Genta 100, 100 mg/ml bei 35 Pferden) oder einer Konservierungsstoff freien Gentamicin Injektion (0,05 ml, 80 mg/ml bei 52 Pferden) unter Sedation und lokaler Anästhesie behandelt.

Ergebnisse: Subkonjunktivale Blutungen (26/86; 30,2 %) und intrakamerale Blutungen (4/86; 4,7 %) zählten zu den unmittelbaren Komplikationen. Die Komplikationen nach der Injektion bestanden aus Kataraktbildung oder -reifung (5/59 Augen; 8,5 %) und Netzhautdegeneration (3/59 Augen; 5,1 %). Bei der Mehrzahl der Pferde war ein positiver Therapieerfolg (keine weiteren rezidivierenden oder persistierenden Entzündungen) zu beobachten (52/59 Pferde; 88,1 %). Wiederkehrende Entzündungen wurden in 5/59 (8,5 %) Pferden und persistierende Entzündung in 2/59 (3,4 %) Pferden beobachtet.

Schlussfolgerungen: Die niedrig-dosierte intravitreale Gentamicin Injektion stellt eine vielversprechende Behandlungsmethode für verschiedene Typen und Grade von Uveitiden dar. Der relativ hohe Prozentsatz an positivem Behandlungserfolg verbunden mit nur geringen Komplikationsraten rechtfertigt weitere Untersuchungen.

6 SUMMARY

Britta Maria Fischer

Intravitreal injection of low-dose gentamicin: an alternative method of management for equine recurrent uveitis

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Submitted in May 2020

(52 pages, 18 figures, 13 tables, 110 references)

Keywords: Equine recurrent uveitis – intravitreal injection – Gentamicin – Leptospira – Equine ophthalmology

Objective: To describe the intravitreal gentamicin injection technique, report the effects of the injection on the clinical signs of uveitis and to describe the associated peri-injection (within 24 hours) and post-injection complications (30 to 780 days). Additionally, evaluation of the systemic and ocular *Leptospira* status and its effects on the treatment outcome was performed.

Material and Methods: 86 horses of various ages, breeds, and gender presenting with recurrent or persistent uveitis were treated via intravitreal injection of 4 mg of undiluted gentamicin (0.04 ml, Genta 100, 100mg/ml in 35 horses) or preservative-free gentamicin (0.05 ml, 80mg/ml in 52 horses) under sedation and local anesthesia. All 86 horses were observed for complications, and 59 horses were evaluated for their response to therapy (follow-up: 30 to 780 days).

Results: Peri-injection complications consisted of subconjunctival (26/86; 30.2%) or intracameral hemorrhage (4/86; 4.7%). Post-injection complications consisted of cataract formation/maturation (5/59 horses, 8.5%) and retinal degeneration (3/59 eyes 5.1%). The majority of horses 52/59 (88.1%) had a positive clinical outcome (absence of recurrent or persistent inflammation). Recurrent inflammation was documented in 5/59 (8.5%) horses and persistent inflammation was diagnosed in 2/59 (3.4%) horses.

Conclusion: Intravitreal injection of low-dose gentamicin shows promise at controlling different types and stages of uveitis. The relatively high rate of success and low complication rate warrants further investigation.

7 REFERENCES

Abrams KL and Brooks DE. Equine recurrent uveitis: current concepts in diagnosis and treatment. Equine Pract. 1990; 12: 27-35.

Adler B and De Pen A. 2010. Leptospira and leptospirosis. Vet Microbiol. 2010; 140: 287-96.

Ahmad SN, Shah S and Ahamd FM. Laboratory diagnosis of leptospirosis. J Postgrad med. 2005; 51: 195-200.

Allbaugh RA. Equine recurrent uveitis: A review of clinical assessment and management. Equine Vet Educ. 2017; 29: 279-88.

Angelos JA, Oppenheim Y, Rebhun W, Mohammed H, and Antcak DF. Evaluation of breed as a risk factor for sarcoid and uveitis in horses. Anim. Genets. 1988;19: 417–25.

Baath J, Ells AL, Crichton A, Kherani A and Williams RG. Safety profile of intravitreal triamcinolone acetonide. J Ocul Pharmacol and Ther. 2007; 3: 304–10.

Babudieri B. Laboratory diagnosis of Leptospirosis. 1961; 24: 45-58.

Barnett KC. Equine periodic ophthalmia: a continuing aetiological riddle. Equine Vet J. 1987; 19: 90–1.

Blogg JR, Barton MD, Graydon R and Cust RE. 2010. Blindness caused by rhodococcus equi infection in a foal. Equine Vet J. 1983; 15: 25–6.

Båverud V, Gunnarsson A, Olsson Engvall E, Franzén P, and Egenvall A. Leptospira seroprevalence and associations between seropositivity, clinical disease and host factors in horses. AVS. 2009; 51: 15.

Bellone RR. Genetic testing as a tool to identify horses with or at risk for ocular disorders. Vet Clin North Am Equine Pract. 2017; 33: 627-45.

Braga J, Hamond C, Martins G, Neves Abreu R and Lilenbaum W. Ophthalmic alterations in horses with leptospirosis by serovar icterohaemorrhagiae in Rio de Janeiro, Brazil. Pesq Vet Brasil. 2011; 31.

Brandes K, Wollanke B, Niedermaier G, Brem S Gerhards H. 2007. Recurrent uveitis in horses: vitreal examinations with ultrastructural detection of leptospires. J Vet

Med A Physiol Pathol Clin Med. 2007; 54: 270–5.

Brooks DE, Matthews A and Clode A. Diseases of the Cornea. In: Gilger BC, Hrsg. Equine Ophthalmology. 3. Aufl. Iowa: Wiley Blackwell; 2016. p. 252-68.

Cole JR, Sulzer CR and Pursell AR. Improved microtechnique for the leptospiral microscopic agglutination test. Appl Microbiol. 1973; 25: 976-80.

Davidson MG. Anterior uveitis. In: Robinsons, NE, Hrsg. Current therapy in equine medicine. 3. Aufl.. Philadelphia: WB Saunders; 1992. p. 593–4.

Deeg CA, Kaspers B, Gerhards H, Thurau SR, Wollanke B and Wildner G. Immune responses to retinal autoantigens and peptides in equine recurrent uveitis. Investig Ophthalmol Vis Sci. 2001; 42: 393–8.

Deeg CA, Ehrenhofer M, Thurau SR, Reese S, Wildner G, and Kaspers B. Immunopathology of recurrent uveitis in spontaneously diseased horses. Exp Eye Res. 2002; 75: 127–33.

Deeg CA, Thurau SR, Gerhards H, Ehrenhofer M, Wildner G and Kaspers B. Uveitis in horses induced by interphotoreceptor retinoid-binding protein is similar to the spontaneous disease. Eur J Immunol. 2002; 32: 2598–606.

Deeg CA, Marti E, Gaillard C and Kaspers B. Equine recurrent uveitis is strongly associated with the MHC class I haplotype ELA-A9. 2004; 36: 73–5.

Deeg CA, Reese S, Gerhards H, Wildner G and Kaspers B. The uveitogenic potential of retinal S-antigen in horses. IOVS. 2004; 45: 2286-92. DOI: 10.1167/iovs.03-1226

Deeg CA, Amann B, Raith AJ and Kaspers B. Inter- and intramolecular epitope spreading in equine recurrent uveitis. IOVS. 2006; 47: 652-6. DOI: 10.1167/iovs.05-0789

Deeg CA, Pompetzki D, Raith AJ, Hauck SM, Amann B, Suppmann S and Goebel TWF, Olazabal U, Gerhards H, Reese S, Stangassigner M, Kaspers B and Ueffing M. Identification and functional validation of novel autoantigens in equine uveitis. Mol. Cell Proteomics . 2006; 5: 1462–70.

Deeg CA,Hauck SM, Amann B, Kremmer E, Stangassinger M and Ueffing M. Major retinal autoantigens remain stably expressed during all stages of spontaneous

uveitis. Mol Immunol. 2007; 44: 3291-6.

Deeg CA, Hauck SM, Amann B, Pompetzki D, Altmann F, Raith A, Schmalzl T, Stangassinger M and Ueffing M. Equine recurrent uveitis - a spontaneous horse model of uveitis. Ophthalmic Res. 2008; 40: 151-3.

Deeg CA, Wildner G and Thurau S. Uveitis in horses, rats and man: what do we learn from our pets? Curr Immunol Rev. 2011; 7: 368-77.

Degenring RF and Jonas BJ. Intravitreal injection of triamcinolone acetonide as treatment for chronic uveitis. Br J Ophthalol. 2003; 87: 361.

Diedrichs-Mohring M, Leban J, Strobl S, Obermayr F and Wildner G. A new small molecule for treating inflammation and chorioretinal neovascularization in relapsing-remitting and chronic experimental autoimmune uveitis. IOVS. 2015; 56: 1147–57. DOI: 10.1167/iovs.14-15518

De Groot-Mijnes JD, Rothova A, van Loon AM, Schuller M, ten D-VLNH, de BJH, Schuurman R, Weersink AJL. Polymerase chain reaction and Goldmann-Witmer coefficient analysis are complimentary for the diagnosis of infectious uveitis. Am J Ophthalmol. 2006; 141: 313–8.

Douglas LC, Yi NY, Davis JL, Salmon JH, Gilger BC. Ocular toxicity and distribution of subconjunctival and intravitreal rapamycin in horses. J Vet Pharmacol Ther. 2008; 31: 511–6.

Dwyer AE, Crockett RS and Kalsow CM. Association of leptospiral seroreactivity and breed with uveitis and blindness in horses: 372 cases (1986-1993). J Am Vet Med Assoc. 1995; 207: 1327–31.

Ellis WA. The diagnosis of leptospirosis in farm animals. In: Ellis WA and Little TWA, Hrsg. The present state of leptospirosis diagnosis and control. 1. Aufl.. Dordrecht, Netherlands: Martinus Nijhoff Publishers; 1986. p. 13-32.

Faber NA, Crawford M, LeFebvre RB, Buyukmihci NC, Madigan JE and Willits NH. Detection of leptospira spp. in the aqueous humor of horses with naturally acquired recurrent uveitis. J Clin Microbiol. 2000; 38: 2731–3.

Faine S. Guidelines for the control of leptospirosis. 1982. WHO Offset Publication No. 67, WHO, Geneva

Faine S, Adler B, Perolat P and Bolin C. Leptospira and Leptospirosis. 2 Aufl.. Melbourne: MediSci; 1999.

Featherstone HJ and Heinrich CL. Ophthalmic examination and diagnostics. In: Gelatt KN, Gilger BC and Kern TJ, Hrsg. Veterinray Ophthalmology. 5. Aufl.. Iowa: Wiley-Blackwell; 2013. p. 533-613.

Fischer BM, McMullen RJ, Reese S. and Brehm W. Intravitreal injection of low-dose gentamicin for the treatment of recurrent or persistent uveitis in horses: Preliminary results. BMC Vet Res. 2019; 15: 29. DOI: 10.1186/s12917-018-1722-7

Fritz KL, Kaese HJ, Valberg SJ, Hendrickson JA, Rendahl AK, Bellone RR, Dynes KM, Wagner ML, Lucio MA, Cuomo FM, Brinkmeyer-Langford CL, Skow LC, Mickelson JR, Rutherford MS, McCue ME. Genetic risk factors for insidious equine recurrent uveitis in appalosa horses. Anim. Genet. 2014; 45: 392–9.

Frühauf B, Ohnesorge B, Deegen E and Boevé M. Surgical management of equine recurrent uveitis with single port pars plana vitrectomy. Vet Ophthalmol. 1998; 1: 137–51.

Gilger BC, Malok E, Cutter KV, Stewart T, Horohov DW and Allen JB. Characterization of T-lymphocytes in the anterior uvea of eyes with chronic equine recurrent uveitis. Vet Immunol Immunop. 1999; 71: 17-28.

Gilger BC, Michau TM. Equine recurrent uveitis: New methods of management. Vet Clin North Am - Equine Pract. 2004; 20: 417–27.

Gilger BC, Salmon JH, Wilkie DA, Cruysberg LPJ, Kim J, Hayat M, Kim H, Kim S, Yuan P, Harrington SM, Murray PR, Edelhauser HF, Csaky, KG and Robinson, MR. 2006. A novel bioerodible deep scleral lamellar cyclosporine implant for uveitis. IOVS. 2006; 47: 2596–605. DOI: 10.1167/iovs.05-1540.

Gilger BC, Salmon JH, Yi NY, Barden CA, Chandler HL, Wendt JA and Colitz CMH. Role of bacteria in the pathogenesis of recurrent uveitis in horses from the southeastern united states. AVMA. 2008; 69: 1329–35.

Gilger BC, Wilkie DA, Clode AB, McMullen RJ, Utter ME, Komaromy AM, Brooks DE, Salmon JH. Long-term outcome after implantation of a suprachoroidal cyclosporine drug delivery device in horses with recurrent uveitis. Vet Ophthalmol.

2010; 13: 294–300.

Gilger BC. Equine recurrent uveitis: the viewpoint from the USA. Equine Vet J. 2010; 42: 57–61.

Gilger BC and Hollingsworth SR. Equine recurrent uveitis. In: Gilger BC, Hrsg. Equine ophthalmology, 3. Aufl. Missouri: Elsevier Science; 2010. p. 369–415.

Gilger BC, Abarca EM, Salmon JH and Patel S. Treatment of acute posterior uveitis in a porcine model by injection of triamcinolone acetonide into the suprachoroidal space using microneedles. IOVS. 2013; 54: 2483–92. DOI: 10.1167/iovs.13-11747.

Gerding JC, Gilger BC. Prognosis and impact of equine recurrent uveitis. Equine Vet J. 2016; 48: 290–8.

Goldmann HA and Witmer R. Antikörper im Kammerwasser. Ophthalmologica. 1954; 127: 323–30.

Grahn BH and Cullen CL. Equine phacoclastic uveitis: the clinical manifestations, light microscopic findings, and therapy of 7 cases. Can Vet J. 2000; 41: 376-82.

Gussenhoven GC, Horn MAWGVD, Goris MGA, Terpstra WJ, Hartskeerl RA, Mol BW, Ingen CWV and Smith HL. Lepto dipstick, a dipstick assay for detection of Leptospira.specific immunoglobulin M antibodies in human sera. J Clin Microbiol. 1997; 35: 92-7.

Halliwell RE, Brim TA, Mines MT, Wolf D and White FH. Studies on equine recurrent uveitis. II: The role of infection with leptospira interrogans serovar pomona. Curr Eye Res. 1985; 4: 1033–40.

Hartskeerl RA, Goris MGA, Brem S, Meyer P, Kopp H, Gerhards H and Wollanke B. Classification of leptospira from the eyes of horses suffering from recurrent uveitis. J Vet Med B Infect Dis Vet Public Health. 2004; 51: 110–5.

Heusser H. Die periodische Augenetzündung- eine Leptospirose? SAT. 1984; 90: 288–312.

Holländer GA, Fruman DA, Bierer BE and Burakoff SJ. Disruption of T Cell development and repertoire selection by calcineurin inhibition in vivo. Transplant. 1994; 58: 1037-43.

Houwers DJ, Goris MG, Abdoel T, Kas JA, Knobbe SS, van Dongen AM, Westerduin FE, Klein WR and Hartskeerl RA. Agglutinating antibodies against pathogenic leptospira in healthy dogs and horses indicate common exposure and regular occurrence of subclinical infections. Vet Microbiol. 2011; 148: 449-51.

Jonas JB. Intravitreal triamcinolone acetonide: a change in a paradigm. Ophthalmic Res. 2006; 38: 218-45.

Kalsow CM and Dwyer AE. Retinal immunopathology in horses with uveitis. Ocul Immunol Inflamm. 1998; 6: 239–51.

Kay JE. Inhibitory effects of cyclosporin A on lymphocyte activation. In: Thomason AW, Hrsg. Cyclosporin. 1. Aufl. Dordrecht: Springer; 1989. p 1–23.

Kermani-Arab V, Salehmoghaddam S, Danovitch G, Hirji K and Rezai A. Mediation of the antiproliferative effect of cyclosporine on human lymphocytes by blockade of interleukin 2 biosynthesis. Transplant. 1985; 39: 439-42.

Kleinpeter A, Pohlmann A, Mütze M. Intravitreale Gentamicin-Injektion zur Therapie der equinen rezidivierenden Uveitis- Methode und Fallauswertung. Leipzig: Leipziger Tierärztekongress; 2014. p. 27–30.

Kleinpeter A, Göpfert A, Köhler E and Brehm W. Intravitreale Low-Dose-Gentamicininjektion Zur Behandlung ERU-erkrankter Pferde. Tierärztl Prax. 2019; 47: 25–34.

Kopf H, Gonzalo M, Howard OMZ and Chen X. Rapamycin inhibits differentiation of Th17 cells and promotes generation of FoxP3+ T regulatory cells. Int Immunophamacol. 2007; 7: 1819-24.

Kulbrock M, Lehner S, Metzger J, Ohnesorge B and Distl O. A genome-wide association study identifies risk loci to equine recurrent uveitis in German warmblood horses. PLOS ONE. 2013; 8: e71619. DOI: 10.1371/journal.pone.0071619.

Launois T, Margarita L, Hilarión G, Barbe F, Leurquin C, Bihin B, Hontoir F, Dugdale A, and Vandeweerd JM. Use of intravitreal injection of gentamicin in 71 horses with equine recurrent uveitis. JEVS. 2019; 77: 93-7. DOI: 10.1016/j.jevs.2019.02.018

Levett PN. Leptospirosis: re-emerging or re-discovered disease? J Med Microbiol. 1999;

48: 417-8.

Levett PN. Leptospira Culture. In: Leber AL, Hrsg. Clinical microbiology procedures handbook. 4. Aufl. New Jersey: John Wiley & Sons; 2016. p. 3.14.1-3.

Lorbeer S. Ein Beitrag Zur Vererbungsfrage der periodischen Augenentzündung des Pferdes [Dissertation med. vet]. Hannover: Tierärtzl. Hochschule; 1940.

Lowe RC. Equine uveitis: a UK perspective. Equine Vet J. 2010; 42: 46–9.

Maggs DJ. Ocular manifestations of equine herpesviruses. In: Robinson NE, Hrsg. Current therapy in equine medicine. 5. Aufl. Philadelphia: Saunders; 2003. p. 2003473–6.

Mair TS and Crispin SM. Immunological mechanisms in uveitis. EVJ. 1989; 21: 391–3.

Malalana F, Blundell RJ, Pinchbeck GL and Mcgowan CM. 2017. The role of *Leptospira* spp. in horses affected with recurrent uveitis in the UK. EVJ. 2017; 49 : 706–9.

McMullen RJ. Intravitreal injection of low-dose gentamicin in horses for treatment of chronic recurrent or persistent uveitis: preliminary results. Proceedings of the Interntaional Equine Ophthalmology Consortium; 2015 June 4-6; Savannah, USA.

McMullen RJ, Fischer BM. Medical and Surgical Management of Equine Recurrent Uveitis. Vet Clin North Am-Equine Pract. 2017; 33: 465–81.

Miller TR, Whitley RD. Uveitis in horses. Mod Vet Pract. 1987; 8: 351–7.

Miller TL, Willis AM, Wilkie DA, Hoshaw-Woodard S, and Stanley JRL. Description of ciliary body anatomy and identification of sites for transscleral cyclophotocoagulation in the equine eye. Vet Ophthalmol. 2001; 4: 183–90.

Mochmann H. Laboratoriumsdiagnose der Leptospirosen und der Leptopsiren. Zbl Bkat Par Inf I Orig. 1963; 192: 385-99.

Morter RL, Herschler RC, Fessler JF and Lavignette A. Experimental equine leptospirosis (Leptospira pomona). In Proceedings of the 68th Annual Meeting of the US Animal Health Association; 1964; Memphis, TN, USA. Richmond, VA, USA: United States Animal Health association; 1964.

OIE 2.1.9.- Leptospirosis. In: OIE Terrestrial mannual. 2008. p. 251-64.

Parma AE, Santisteban CG, Villalba JS and Bowden RA. Experimental demonstration of an antigenic relationship between Leptospira and equine cornea. Vet Immunol Immunopathol. 1985; 10: 215–24.

Parma AE, Fernandez AS, Santisteban CG, Bowden RA and Cerone SI. Tears and aqueous humor from horses inoculated with Leptospira contain antibodies which bind to cornea. Vet Immunol Immunopathol. 1987; 14: 181–5.

Parma AE, Cerone SI and Sansinanea SA. Biochemical analysis by SDS-PAGE and western blotting of the antigenic relationship between Leptospira and equine ocular tissues. Vet Immunol Immunopathol. 1992; 33: 179–85.

Pinard C. Gentamicin injection. Proceedings of the 36th annual meeting of the American College of Veterinary Ophthalmologists; 2005 Oct 12-15; Nashville, Tennessee, USA. Vet Ophthalmol; 2005.

Pinto NI, McMullen RJ, Linder KE, Cullen JM, Gilger BC. Clinical, histopathological and immunohistochemical characterization of a novel equine ocular disorder: Heterochromic iridocyclitis with secondary keratitis in adult horses. Vet Ophthalmol. 2015; 18: 443–56.

Priest HL, Irby NL, Schlafer DH, Divers TJ, Wagner B, Glaser AL, Chang JF and Smith MC. Diagnosis of Borrelia-associated uveitis in two horses. Vet Ophthalmol. 2012; 15: 398–405.

Rebhun WC. Diagnosis and treatment of equine uveitis. J Am Vet Med Assoc. 1979; 175: 803–8.

Regan DP, Aarnio MC, Davis WS, Carmichael KS, Vandenplas ML, Lauderdale JD and Moore PA. Characterization of cytokines associated with Th17 cells in the eyes of horses with recurrent uveitis. Vet Ophthalmol. 2012; 15: 145–52.

Rimpau W. 1947. Leptospirose beim Pferd (Periodische Augenentzündung). Tierärtzl Umschau. 1947; 20: 15–6.

Roberts SR. Chorioretinitis in a band of horses. JAVMA. 1971; 158: 2043–6.

Rohrbach BW, Ward DA, Hendrix DVH, Cawrse-Foss M and Moyers TD. Effect of vaccination against Leptospirosis on the frequency, days to recurrence and

progression of disease in horses with equine recurrent uveitis. Vet Ophthalmol. 2005; 8: 171–9.

Romeike A, Brügmann M, Drommer W. Immunohistochemical studies in equine recurrent uveitis (ERU). Vet Pathol. 1998; 35: 515–26.

Sandmeyer LS, Bauer BS and Feng CX. Equine recurrent uveitis in western Canadian prairie provinces: a retrospective study (2002-2015). Can Vet J. 2017; 58: 717-22.

Sauvage AC, Monclin SJ, Elansary M, Hansen P and Grauwels MF. Detection of intraocular Leptospira spp. by real-time polymerase chain reaction in horses with recurrent uveitis in Belgium. Equine Vet J. 2019; 51: 299-303.

Schwink KL. Equine Uveitis. Vet Clin North Am - Equine Pract. 1992; 8: 557–74.

Spiess BM. Equine recurrent uveitis: the European viewpoint. Equine Vet J. 2010; 42: 50–6.

Sponenberg DP and Bellone R. Equine color genetics. 4.Aufl. New Jersey: Wiley Blackwell; 2017

Swadzba ME, Hauck SM, Naim HY, Amann B and Deeg CA. Retinal glycoprotein enrichment by concanavalin a enabled identification of novel membrane autoantigen synaptotagmin-1 in equine recurrent uveitis. PLOS ONE. 2012; 7: e50929. DOI: 10.1371/journal.pone.0050929

Tömördy E, Hässig M, Spiess BM. The outcome of pars plana vitrectomy in horses with equine recurrent uveitis with regard to the presence or absence of intravitreal antibodies against various serovars of Leptospira interrogans. Pferdeheilk. 2010; 26: 251–4.

Trepanier DJ, Gallant H, Legatt DF and Yatscoff RW. Rapamycin: distribution, pharmacokinetics and therapeutic range investigations: an update. Clin Biochem. 1998; 31: 345-51.

Verma A, Kumar P, Babb K, Timoney JF and Stevenson B. Cross-reactivity of antibodies against leptospiral recurrent uveitis-associated proteins A and B (LruA and LruB) with eye proteins. PLoS Negl Trop Dis. 2010; 4: e778. DOI: 10.1371/journal.pntd.0000778.

Verma A, Matsunaga J, Artiushin S, Pinne M, Houwers DJ, Haake DA, Stevenson B

and Timoney JF. Antibodies to a novel leptospiral protein, LruC, in the eye fluids and sera of horses with Leptospira-associated uveitis. Clin Vaccine Immuol. 2012; 19: 452-6.

von Borstel M, von Oppen T, Glitz F, Frühauf B, Deegen E, Boevé MH, Ohnesorge B. Langzeitergebnisse der Pars plana Vitrektomie (double port) bei Equiner Rezidivierender Uveitis. Pferdeheilk. 2005; 21: 13–8.

von Borstel M, Oey L, Strutzberg-Minder K, Boeve MH and Ohnesorge B. Direct and indirect detection of leptospiresin vitreal samples of horses with ERU. Pferdeheilk. 2010; 26: 219-25.

Werry H and Honegger H. Pars-plana vitrektomie bei chronischer uveitis. Klin Monatsbl Augenheilk. 1987; 191: 9–12.

Werry H, Gerhards H. Zur operativen Therapie der equinen rezidivierenden Uveitis (ERU). Tierärztl Prax. 1992; 20: 178–86.

Wildner G and Kaufmann U. What causes relapses of autoimmune diseases? The etiological role of autoreactive T Cells. Autoimmun Rev. 2013; 12: 1070-5.

Winterberg A, Gerhards H. Langzeitergebnisse der Pars-plana-Vitrektomie bei equiner rezidivierender Uveitis. Pferdeheilk. 1997; 13: 377–83.

Wiesner E and Ribbeck R. Lexikon der Veterinärmedizin. Stuttgart: Enke im Hippokratesverlag GmbH; 2000.

Wollanke B, Rohrbach BW, Gerhards H. Serum and vitreous humor antibody titers in and isolation of Leptospira interrogans from horses with recurrent uveitis. J Am Vet Med Assoc. 2001; 219: 795–800.

Yi NY, Davis JL, Salmon JH, Gilger BC. Ocular distribution and toxicity of intravitreal injection of triamcinolone acetonide in normal equine eyes. Vet Ophthalmol. 2008; 11: 15–9.

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