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Case Report

Outcome of superficial keratectomy without conjunctival graft as a surgical technique for immune-mediated keratitis in horses: Four cases

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Summary

This case report describes four horses with unilateral superficial or mid-stromal immune-mediated keratitis (IMMK) treated with a superficial keratectomy (SK) without a conjunctival graft. In two horses, the surgery was performed under general anaesthesia, and in two horses standing with sedation and local blocks. Results of this report show that SK is a viable treatment option in horses with chronic superficial and/or mid-stromal IMMK that can even be performed in the standing, sedated horse. When sufficient corneal tissue is removed, no recurrence is to be expected in the long-term follow-up (up to 31 months). In two horses, healing occurred without complications. Two horses developed a secondary bacterial infection post-operatively (*Enterococcus faecalis* and *Staphylococcus aureus*). In one case, this resulted in a pre-perforating melting corneal ulcer necessitating conjunctival pedicle graft surgery 13 days post keratectomy. In three horses, there was no recurrence of the IMMK with a long-term follow-up of 6–31 months. One case showed recurrence of IMMK in the cornea region surrounding the keratectomy 9 months after surgery.

Introduction

Immune-mediated keratitis (IMMK) is a common, chronic, noninfectious, inflammatory ocular disease in horses (Brooks et al., 2017). The aetiopathogenesis is presumed to be immune-mediated based on the lack of the detection of aetiologic organisms, a predominant T-cell-mediated infiltrate found on histopathology, and a beneficial response to topical immunosuppressive treatment (Brooks et al., 2017; Gilger & Michau, 2005; Matthews & Gilger, 2009; Pate et al., 2012).

In most cases, diagnosis is based on clinical appearance and beneficial effects of immunosuppressive medication (Brooks et al., 2017; Matthews & Gilger, 2009). Although the clinical appearance is variable, in all cases the following features are present to a varying degree in the cornea: oedema, neovascularisation, cellular infiltrate, disruption of epithelium, epithelial hyperplasia, superficial and stromal pigmentation, transient epithelial bullae, intrastromal haemorrhage, ghost vessels, stromal fibrosis and hyperaemia of the conjunctiva (Brooks et al., 2017).

Clinical presentations of IMMK have been categorised into three main groups according to the location of the primary inflammatory signs: superficial IMMK (45% of cases), mid-deep stromal (27% of cases) and endothelial (23% of cases), then epithelial and eosinophilic, the latter two being much less common (Matthews & Gilger, 2009). Response to treatment of IMMK differs somewhat based on the subgroup, but the mainstay of topical medical therapy is based on immunosuppression, namely, topical glucocorticoids, Cyclosporin A (CsA) and/or Tacrolimus (Gratzek et al., 1995; Matthews, 1995; Matthews & Gilger, 2009). Medical treatment is required for a long period of time and sometimes even lifelong. Surgical treatment options include episcleral implantation of silicone matrix CsA delivery devices (Gilger et al., 2014), superficial keratectomy (SK) (with/without a pedicle/amnion graft), lamellar corneal graft, penetrating keratoplasty and subconjunctival bone marrow-derived mesenchymal stem cell therapy (Davis & Schnabel, 2019; Gilger et al., 2005).

To the authors' knowledge, long-term follow-up of SK without a conjunctival graft, and performed in the standing horse, has not yet been documented. In this case series, the outcome of four horses with unilateral IMMK that were treated with SK is described (Gilger et al., 2005). In this report, 'SK' reflects to SK without conjunctival graft.

Case history and clinical findings

The four horses included in this study were examined at the Department of Clinical Sciences of Utrecht University. Ophthalmic examination included neuro-ophthalmic testing, diffuse and focal direct illumination (Pneumatic Otoscope, Beta 100, Heine), hand-held slit-lamp bio microscopy (SL-15, Kowa), rebound tonometry (Tonovet tonometer) and sterile dye strips (fluorescein [BioGlo, Henry Schein], and rose Bengal [Optitech Eyecare]).

Case history

Three geldings and one mare (mean age 11 years, range 8–14 years) were included in this study. The horses in this case series are referred as Case 1, Case 2, Case 3 and Case 4. Patient information of the four cases is summarised in **Table 1**. All horses were presented to the equine clinic of Utrecht University for recurring unilateral corneal problems lasting 5 months to 2 years. The recurrent complaints included signs of

discomfort, corneal oedema, corneal opacifications, ulcerations and neovascularisation. Topical treatment prescribed by the referring veterinarians varied between cyclosporin (2%, 20 mg/ml Ciclosporine, AST beheer BV), chloramphenicol (4 mg/g, Cavasan, AST beheer BV), dexamethasone (0.1%, 1 mg/mg, Dexamethasone, TEVA), oxytetracycline (5 mg/g) plus polymyxin B (10,000 IE/g) (Terramycin, Zoetis), gentamicin (3 mg/ml) plus polymyxin B (10,000 IE/g) (Gentapol B 5 ml, AST beheer BV).

Clinical findings

Ophthalmic examination showed mild blepharospasm (n = 1), corneal oedema (n = 4) with (n = 3, see **Fig 1b-d**) or without (n = 1) neovascularisation (**Fig 1d**), calcifications (n = 2; **Fig 1a**), bullous corneal opacity (n = 1; **Fig 1d**) and fluorescein uptake (n = 1). Rebound tonometry measurements (in mmHg) were within normal limits in all horses (reference values 16–30 mmHg).

In Case 1 with corneal ulceration, cytology was performed: the cornea showed one cytoplasmatic inclusion in an epithelial cell, and no eosinophils or fungal hyphae were detected. In Case 3, ultrasonographic examination (Linear, 18–5 MHz, Epiq 5, Philips Nederland BV Healthcare) was performed; focal corneal thickening/oedema was seen.

Based on the clinical findings in all cases, a presumptive diagnosis of superficial or mid-stromal IMMK was made (see **Fig 1**).

Treatment

Topical treatment after initial presentation differed between the horses and included CsA (2%, Ciclosporine 20 mg/ml, AST beheer BV) (n = 4), dexamethasone (0.1%, 1 mg/mg, Dexamethason, TEVA) (n = 2), gentamicin (3 mg/ml) plus polymyxin B (10,000 IE/g) (Gentapol B 5 ml, AST beheer BV) (n = 1) and trifluridine (10 mg/ml, Virophta, Horus pharma) (n = 1). Gentamicin (3 mg/ml) plus polymyxin B (10,000 IE/g) (Gentapol B 5 ml, AST beheer BV) and trifluridine (10 mg/ml, Virophta, Horus pharma) were used in Case 1 with a fluorescein positive defect of the cornea and a cytoplasmatic inclusion in an epithelial cell found on cytology. This horse was initially suspected of a viral keratitis. In all horses, topical treatment was eventually started with CsA (2%, 20 mg/ml, Ciclosporine, AST beheer BV) 3 times daily and tapered gradually. In Cases 2 and 4, improvement was seen initially with CsA (2%, 20 mg/ml, Ciclosporine, AST beheer BV) treatment, but during tapering of the medication the signs recurred. In Case 3, no improvement was seen after 8 weeks of topical CsA (2%, 20 mg/ml, Ciclosporine, AST beheer BV), and medication was switched to topical dexamethasone (0.1%, 1 mg/mg, Dexamethason, TEVA) 3 times daily. Although improvement with dexamethasone (0.1%, 1 mg/mg, Dexamethason, TEVA) was seen in this case initially, the clinical signs recurred after discontinuation of the treatment. In Cases 2, 3 and 4, an SK was advised to the owners because no long-term improvement was seen with topical medication.

Case 1 did show beneficial effects from CsA (2%, 20 mg/ml, Ciclosporine, AST beheer BV), and received four episcleral silicone matrix CsA delivery implants (Pharmacy, North Carolina State University) (Gilger et al., 2014). Post-operatively, topical CsA (2%, 20 mg/ml, Ciclosporine, AST beheer BV) treatment was continued (twice daily). Ten days after surgery, blepharospasm and ocular discharge occurred. Topical dexamethasone (0.1%, 1 mg/mg, Dexamethason, TEVA) once daily was added, and the topical therapy was tapered down, and eventually stopped after 2 months. The eye remained stable without treatment during the next 7 months. After 7 months, the horse was admitted again because of ocular discomfort. The corneal oedema had progressed, and a corneal calcification had developed (see **Fig 1a**). Due to the corneal calcifications and recurrent ulcerations, discontinuation of topical treatment was indicated, and a SK was advised to the owner.

Surgery

In Cases 1 and 2, surgery was performed with the horse under general anaesthesia in lateral recumbency. Surgery in Cases 3 and 4 was performed in the standing sedated horse. In the horses under general anaesthesia, premedication consisted of detomidine hydrochloride (0.01 mg/kg bwt intravenous i.v., 7.6 mg/ml, Domosedan, Vetoquinol BV) and butorphanol (0.02 mg/kg bwt i.v., 10 mg/ml, Dolorex, Intervet International BV). In the standing sedated horses, morphine (0.1 mg/kg bwt i.v., 10 mg/ml, Centrafarm) instead of butorphanol (Dolorex, Intervet International BV) was used to eliminate head bobbing and hypersensitivity as described side effects of butorphanol (Potter et al., 2016). All medication was administered through a jugular catheter (Intraflon 12G × 80 mm). All horses also received meloxicam (0.6 mg/kg btw i.v., 20 mg/ml, Metacam, Boehringer Ingelheim), benzylpenicillin (20,000 IE/kg bwt i.v., Benzylpenicilline Natrium, Dechra Pharmaceuticals) and gentamicin (6.6 mg/kg bwt i.v., 50 mg/ml, Gentamycine 5%, Dechra Pharmaceuticals). The eyelashes were clipped, and the conjunctival sac and nasolacrimal duct were flushed with 0.5% iodine solution (100 mg/ml, Betadine, Mylan BV). The cornea was instilled with topical tetracaine HCL (0.5%, 10 mg/ml, Bausch & Lomb).

General anaesthesia was induced with midazolam (0.05 mg/kg bwt i.v., 5 mg/ml, Aurubindo Pharma BV) and

TABLE 1: Age, gender, breed, duration of complaints before superficial keratectomy (SK) in months, area of the keratectomy site and follow-up period in months of the four cases (3A; first SK and 3B second SK)

	Age (year)	Gender	Breed	Duration complaints (months)	Area of the SK	Follow-up (months)
Case 1	8	Mare	DWB	31	50% of the cornea	31
Case 2	13	Gelding	DWB	26	1 × 1 cm	15
Case 3A	9	Gelding	Friesian	23	1 × 1 cm	15
Case 3B	See 3A	See 3A	See 3A	Recurrence after 9	70% of the cornea	6
Case 4	14	Gelding	DWB	20	1 × 1 cm	6

DWB, Dutch Warmblood.

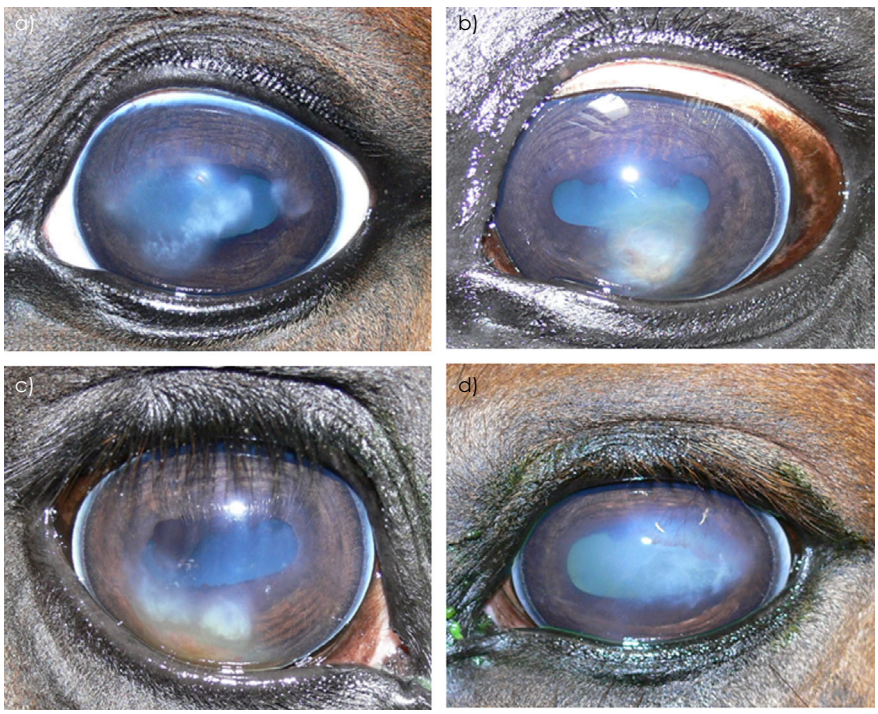

Fig 1: Ophthalmic photographs of all four horses before surgery. (a) Case 1 showing dense corneal oedema centrally, one ghost vessel at the dorsomedial corneal quadrant and minor superficial calcification of the ventral corneal quadrant. (b) Case 2 showing ventral and central corneal oedema with superficial neovascularisation of the ventral corneal quadrants. (c) Case 3 showing dense corneal oedema and superficial neovascularisation of the ventral corneal quadrants with moderate superficial stromal cellular infiltrate of the anterior stroma at the proximal end of the blood vessels. (d) Case 4 showing fluorescein negative bullous corneal opacities dorsolateral and neovascularisation at the dorsolateral corneal quadrant.

ketamine (2 mg/kg bwt i.v., 100 mg/ml, Narketan, Vetoquinol BV), and the horses were kept under general anaesthesia with isoflurane 1–2% via an endotracheal tube. Detomidine hydrochloride (0.01 mg/kg bwt/h i.v., 7.6 mg/ml, Domosedan, Vetoquinol BV) was used as a constant rate infusion.

In the standing procedure, the following local blocks using mepivacaine (20 mg/ml, Mepidor, AST Farma BV) were placed: retrobulbar (200 mg), auriculopalpebral (40 mg), zygomaticus (40 mg) and subconjunctival (4 mg per site) block (Hermans et al., 2019; Jinks et al., 2018). Standing sedation was provided by constant rate infusion of detomidine hydrochloride (0.01 mg/kg bwt/h i.v., 7.6 mg/ml, Domosedan, Vetoquinol BV).

For proper exposure of the cornea a Lieberman V-shaped open wire eyelid speculum was used. Sterile eye drops (Lacriforte, AST Farma BV) were used to hydrate the cornea every 5 min during the procedure.

In the horses under general anaesthesia (Cases 1 and 2), a complete incisional keratectomy was performed and in the standing sedated horses (Cases 3 and 4), a partial incision keratectomy was performed. In the cases in which a complete incisional keratectomy was performed, the portion of the cornea to be removed was outlined with a No 64 Beaver blade (Beaver Miniblade, Becton Dickinson). The edge of the incised cornea was grasped with a Colibri forceps, and lamellar dissection of the entire incised area was performed with a No 64 Beaver blade (Becton Dickinson) (Annear & Peterson-Jones, 2019; Bosch & Klein, 2005; Brooks et al., 2017; Matthews & Gilger, 2009; Scherrer & Lassaline, 2017).

In the cases in which a partial incision keratectomy was performed, a small corneal incision was made with a fixed-depth microsurgical BeaverGuard guarded 35 mm Depth Blade (Becton Dickinson) or no 64 Beaver blade (Becton Dickinson) adjacent to the affected cornea. A Colibri forceps was used to grasp the incised edge of the cornea, and lamellar dissection was performed with a Martinez lamellar corneal dissector. Following complete lamellar dissection of the involved corneal tissue, corneal section scissors, inserted through the initial incision, were used to complete the keratectomy.

In both techniques, the depth of the initial incision was aimed at 30% of the total corneal thickness. When SK is performed in the standing horse, a partial incision keratectomy is preferred over a complete incisional keratectomy. With the former technique, there is a lower risk of penetrating more than 30% of the corneal depth compared with a complete incisional keratectomy because the procedure only necessitates a small initial incision and is otherwise mainly performed by blunt dissection (Brooks et al., 2017). This results in a lower risk of penetrating the cornea if the horse moves its head unexpectedly. Using a fixed-depth surgical blade for the initial sharp incision helps to stay at the superficial level of the cornea. Complete incisional keratectomy is performed by sharp dissection of the complete outlining of the portion of the cornea to be excised.

In Cases 1, 3 and 4, a subpalpebral lavage system (SPL) (Equivet, Jorgen Kruuse A/S) was placed in the lower eyelid at the medial canthus post-operatively. In Cases 1, 2 and 3, a

temporary complete tarsorrhaphy was placed for protection of the globe during the post-operative period. The keratectomy specimens were placed in a 10% neutral-buffered formalin solution and submitted for histopathology.

Post-operative treatment

All horses were topically treated with gentamicin (3 mg/ml, 3000 IU/ml, Soligenal, Virbac) (and polymyxin B (10,000 IE/g, Gentapol B 5 ml, AST beheer BV)) (6 times daily), acetylcysteine (10%, 100 mg/ml, Apotheek Diergeneeskunde) (4 to 6 times daily) for 19–21 days and atropine (0.5%, 5 mg/ml, Atropinsulfaat) (once daily) for 5–12 days. In Cases 1, 3 and 4, this occurred via the SPL (Equivet, Jorgen Kruuse A/S) and in Case 2 manually in the lower conjunctival sac. The tarsorrhaphies of Cases 1, 2 and 3 were removed 2 days after surgery. Systemic meloxicam (0.6 mg/kg, 20 mg/ml, Metacam, Boehringer Ingelheim) was given once daily orally for a minimum of 8 days and continued depending on signs of discomfort.

Histopathology

Evaluation of the excised corneal samples revealed similar histopathological changes, although present in a varying amount. The corneal epithelium was intact and of normal thickness in all horses. Incidentally, there was slight swelling of epithelial cells and an occasional apoptotic cell in the basal region of the epithelium. The epithelial basement membrane showed variable thickening, with (Case 1, see **Fig 2a**) or without splitting of the membrane, and in Case 1, focal mineralisation of the subepithelial region was noted (**Fig 2b**). The corneal stroma was characterised by a mononuclear inflammatory infiltrate that mainly consisted of lymphocytes and histiocytes. Furthermore, proliferation of keratocytes was noted in the corneal stroma. Neovascularisation was not a prominent histopathological feature. The inflammatory infiltrate was also characterised by variable necrosis of leukocytes. The corneal sample of Case 4 did not reveal an inflammatory infiltrate and did not show other marked histological lesions. The second keratectomy sample (the details of the outcome are presented below) of Case 3 showed a largely similar inflammatory infiltrate as of Case 3 although localised more superficial in the corneal stroma, and with more extensive necrosis of inflammatory cells (**Fig 2c,d**).

Outcome

Short-term follow-up

In the first days post-operatively, all horses showed mild signs of discomfort. In Cases 2 and 3, there were no complications during the immediate post-operative period. Medication was continued for 13–21 days, and the cornea was fluorescein negative 18–30 days post-operatively .

Cases 1 and 4 developed a secondary corneal infection 6–7 days post-operatively with the following clinical signs: blepharospasm, purulent discharge, progressive corneal oedema, stromal infiltration of leucocytes and a melting aspect of the cornea. Topical gentamicin (3 mg/ml, 3000 IU/

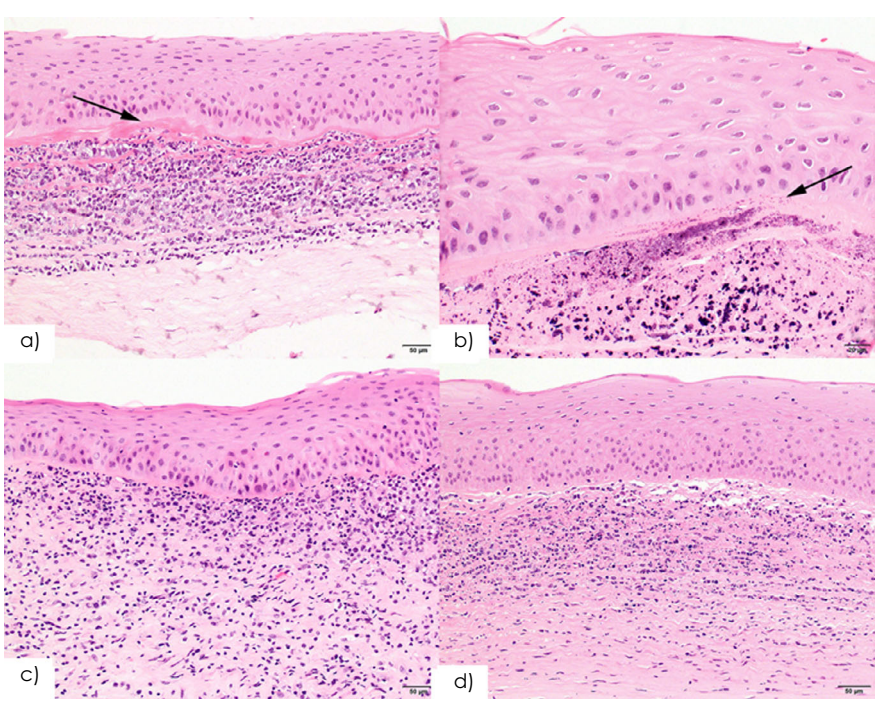

Fig 2: Representative histological pictures of the corneas of the presented cases. (a, Case 1): Equine cornea with intact epithelium showing thickening and splitting of the epithelial basement membrane (arrow). The corneal stroma is characterised by a prominent lymphohistiocytic inflammatory infiltrate. (b, Case 1): Superficial cornea region with marked mineralisation of the basement membrane (arrow) and the subepithelial stroma. Histological images of samples of initial (c, Case 3) and keratectomy 9 months later (d, Case 3) of Case 3. Note that the inflammatory infiltrate in the initial sample is more extensive in the deeper areas of the cornea than in the second sample. All samples are stained with haematoxylin and eosin. Size bar 50 μm, except W (20 μm).

ml, Soligenal, Virbac) was replaced by chloramphenicol (4 mg/g, 4 mg/mg, Cavasan, AST beheer BV) while awaiting the results of the bacterial culture. In Case 1 *Enterococcus faecalis,* and in Case 4 *Staphylococcus aureus* was cultured. In Case 1, topical chloramphenicol (4 mg/g, 4 mg/mg, Cavasan, AST beheer BV) resulted in a comfortable horse after 3 days. Twenty-one days after surgery, complete epithelialisation of the defect was seen, and 3 days later, the treatment was discontinued.

In Case 4, topical chloramphenicol (4 mg/g, 4 mg/mg, Cavasan, AST beheer BV) was replaced with Ofloxacin (0.3%, 3 mg/ml, Trafloxal, Bausch & Lomb) due to resistance of the *S. aureus* against chloramphenicol. Although initially some improvement was seen, a focal area of increased stromal loss developed in the centre of the keratectomy site that necessitated surgery 13 days after the SK (see **Fig 3a**). The horse was brought under general anaesthesia and a conjunctival pedicle graft was placed.

Prior to placing a graft, the centre of the keratectomy site (i.e. the area of the keratectomy site that showed a melting aspect) was debrided using a 6400 Beaver blade. The conjunctival pedicle graft only covered the affected area, the remaining area of the keratectomy site was left uncovered to preserve as much of the visual field as possible. The graft was harvested dorsally and sutured to the cornea with Polyglactin 910 8-0 (Ethicon, Johnson & Johnson) using an interrupted pattern. A temporary tarsorrhaphy was placed using Poliglecaprone 2-0 (Ethilon, Johnson & Johnson). Post-operatively, the pre-operative topical treatment was continued using the SPL (Equivet, Jorgen Kruuse A/S) and tapered down after one week. The horse recovered uneventfully from the second surgery.

Long-term follow-up

For all horses, long-term follow-up was available (6–31 months) by clinical examination, photographs or telephone consultations (see **Table 1**). During this follow-up period, no horse received topical treatment.

In Case 1, the left eye showed moderate fibrosis of the cornea with proper preservation of vision, and no signs of discomfort 2 months post-operatively (see **Fig 4a**). During the follow-up period of 31 months, there was no recurrence of IMMK seen, mild fibrosis of the cornea stayed visible (see **Fig 4b**).

In Case 2, mild focal fibrosis of the SK area developed, and no recurrence of IMMK was seen during the follow-up period of 15 months.

In Case 3, clinical signs recurred 9 months after the SK. Ophthalmic examination at the equine clinic showed mild blepharospasm, corneal oedema, neovascularisation starting from the lateral and medial limbus, marking the lateral aspect of the previous area of the SK and some ghost vessels in the dorsal quadrant. The ventral quadrant (area of the SK) showed moderate fibrosis . A second SK was performed in the standing horse. An upside-down U form shaped area of the cornea was removed around the visible fibrosis of the first keratectomy site, encompassing about 70% of the total corneal surface (see **Fig 5b**). Post-operatively, the horse was treated with the above-mentioned treatment protocol via the SPL (Equivet, Jorgen Kruuse A/S0 [see treatment Case 3]). In the first week, post-operatively the horse showed mild signs of discomfort. After 10 days, the area of the SK was epithelialised, and the horse did not show any signs of

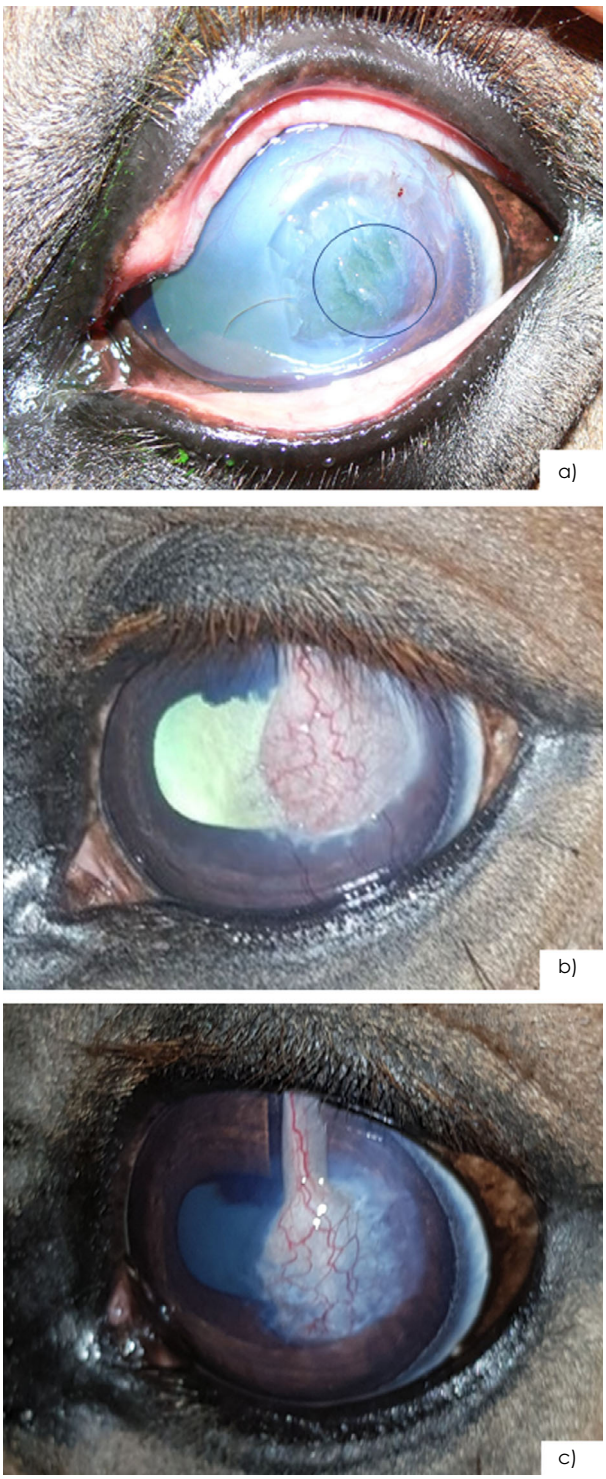

Fig 3: Case 4 before and after placing a conjunctival pedicle graft. (a) Case 4, 10 days after superficial keratectomy (SK) showing diffuse corneal oedema, mainly dorsally and an area of increased stromal loss at the centre of the keratectomy site (black circle). (b) Case 4, 6 weeks after placing a conjunctival pedicle graft showing adherence of the graft, some mild corneal oedema laterally and superficial neovascularisation of the ventral perilimbal area towards the graft. (c) Case 4, 6 months after placing a conjunctival pedicle graft, the horse is comfortable and without topical medication.

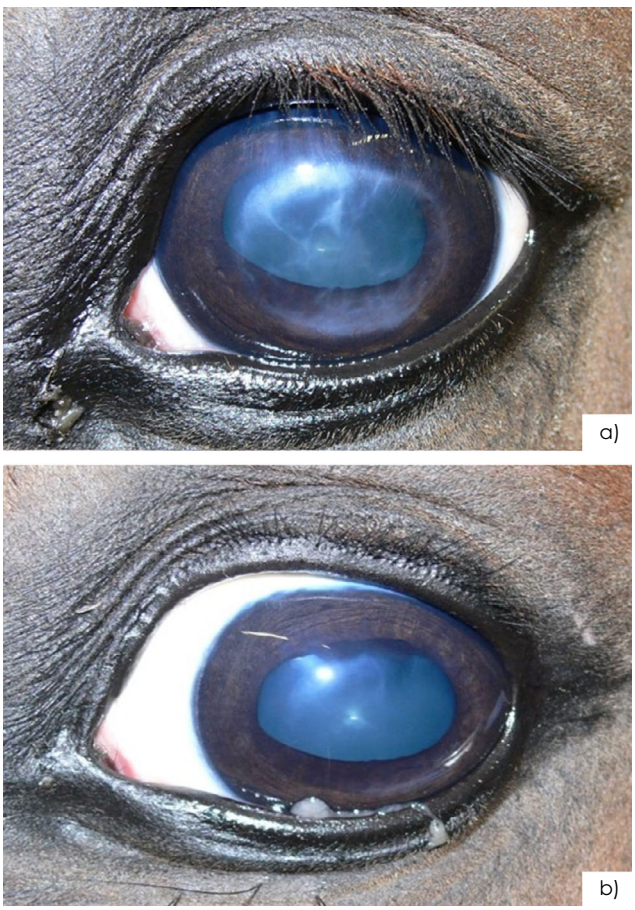

Fig 4: Short-term and long-term follow-up Case 1. (a) Case 1, 2 months after surgery, without topical medication. Moderate fibrosis of the cornea is seen with proper preservation of vision and no signs of discomfort. (b) Case 1, 27 months after superficial keratectomy (SK) showing mild fibrosis at the SK with minimal ocular discharge and no signs of discomfort.

discomfort. Seventeen days after surgery, treatment was discontinued, and no recurrence of IMMK was noted during the follow-up of 6 months. Six months after SK, the eye showed moderate fibrosis at the area of the SK without signs of discomfort (see **Fig 5c**).

In Case 4, after the conjunctival graft surgery, the eye remained comfortable and visual (see **Fig 3c**). Mild fibrosis at the SK area developed around the conjunctival graft. During the follow-up period of 6 months, there was no recurrence of IMMK despite discontinuation of treatment. Because the horse functioned very well with the conjunctival graft and did not seem to have any complaints of a reduced visual field, it was decided to not cut the pedicle of the conjunctival graft.

Discussion

This report documents the outcome of SK as a treatment option for superficial and mid-stromal IMMK in four horses. In two horses, the SK was performed in the standing horse, and in two horses under general anaesthesia. The rationale behind SK is surgical removal of the epithelium and anterior stroma containing the antigen load or sensitised immune cells that are localised within the cornea and that stimulate the inflammatory response (Matthews, 2008; Pate et al., 2012). Altering the local immune environment by surgical intervention could favour reduction of the production of inflammatory mediators, and removal of the antigen load could dampen the immunoinflammatory response (Brooks et al., 2017).

Histopathological findings of Cases 1, 2 and 3 were in agreement with previous reports (Gilger et al., 2005; Matthews & Gilger, 2009), including the presence of stromal fibrosis, infiltration of lymphocytes and macrophages although neovascularisation was not a prominent feature, regardless of the location of IMMK. The corneal sample of Case 4 did not reveal an inflammatory infiltrate and did not show other marked histological lesions. This is in line with the results of the ophthalmic examination the day before SK (one week after discontinuation of the topical treatment with dexamethasone [0.1%, 1 mg/mg, Dexamethason, TEVA]), when no major abnormalities of the cornea were found. In this case, the signs of IMMK were likely still suppressed by the effect of the topical dexamethasone treatment by the time of the SK (Clark, 2007). It could be stated that there should have been a longer time frame between the last treatment with dexamethasone (0.1%, 1 mg/mg, Dexamethason, TEVA) to let the immune suppressive effect of the dexamethasone work out, and to see a clearer border between abnormal and normal corneal tissue.

Outcome of SK as a therapy for IMMK in horses has only been described in cases where SK was directly followed by a grafting procedure (Gilger et al., 2005). In the five horses of Gilger et al. (2005) treated with SK with conjunctival grafting, no complications were described, and despite discontinuation of treatment, no recurrence of IMMK has been noted after surgery (mean follow-up of 13.3 ± 11.5 months after surgery) (Gilger et al., 2005). Although good results were reported by Gilger et al. (2005), it is unknown whether placing a conjunctival graft directly after SK is beneficial or not. A conjunctival graft results in scarring and local loss of corneal transparency that might impair the visual axis. Moreover, it could lead to substantial breakdown of immune privilege of the avascular cornea. Increasing neovascularisation by placing a pedicle graft might even perpetuate an unwanted immune-mediated inflammatory response (Pate et al., 2012).

In this case series, one horse received a conjunctival graft due to melting of the corneal stroma caused by post-operative infection, while in the other three horses the corneas healed well without a graft. This study suggests that some horses do not require a graft, but further study is needed as these case numbers are too low to draw firm conclusions. In the authors' opinion, placing a graft directly following SK is only indicated if the depth of the keratectomy exceeds half the thickness of the cornea unintentionally, if the affected tissue cannot be completely removed, or if other concerns regarding efficacy of the wound healing are present (e.g. deficient tear production or keratomalacia) (Brooks et al., 2017). Benefits of a SK without a conjunctival grafting procedure are reduction of surgical procedure time, easier to perform in the standing horse (which reduces the need of general anaesthesia and related risks) and less visual impairment as created by a conjunctival pedicle graft. Our study shows that about 70% of the corneal surface area can be removed without complications (n = 1).

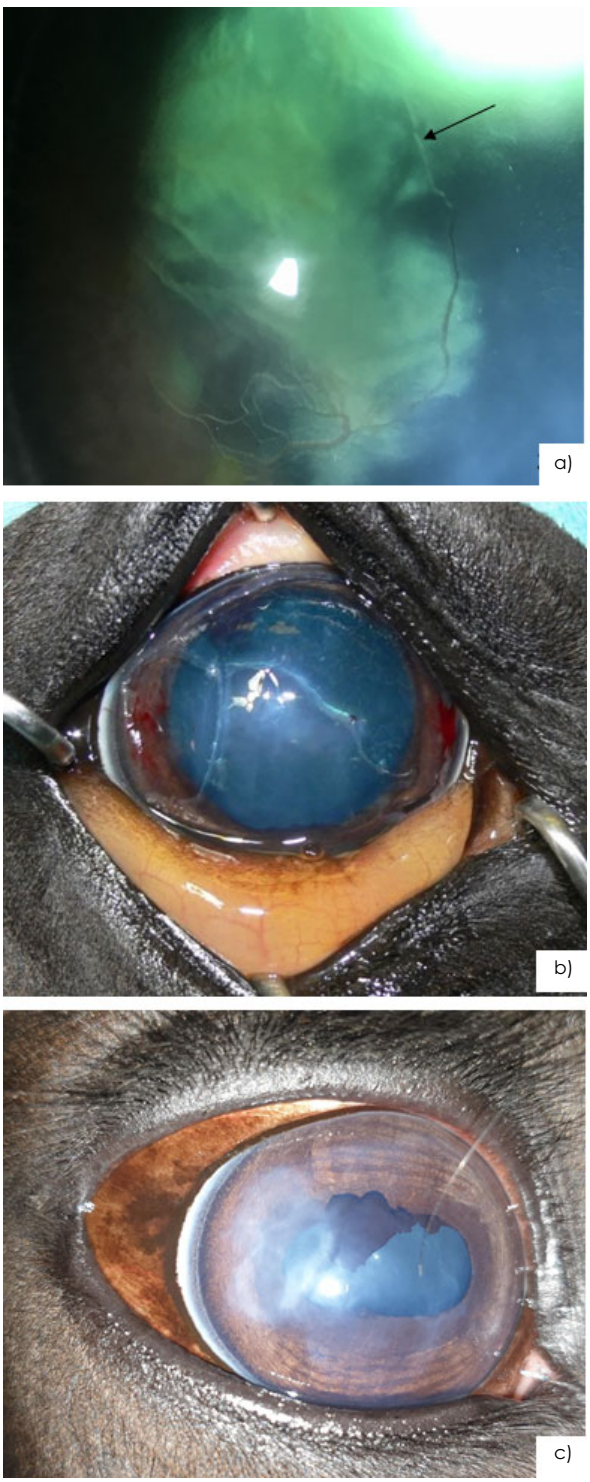

Fig 5: Case 3 before and directly after superficial keratectomy (SK) and 6 months after second surgery. (a) Case 3 before the second SK, showing superficial neovascularisation marking the first keratectomy site. (b) Case 3 directly after SK: An upside-down U-shaped area of the cornea has been removed around the visible fibrosis of the first keratectomy encompassing about 70% of the total corneal surface, some minor/focal bleeding of the cornea is present medially and laterally due to the IMMK related neovascularisation. (c) Case 3, 6 months after the second SK showing moderate fibrosis at the area of the SK.

Depending on the underlying corneal disorder for which a SK is indicated, complications are generally minimal. They may include delayed wound healing, infection, deep stromal involvement, keratomalacia, perforation, recurrence of the original lesion and formation of excessive scar tissue resulting in increased corneal opacification, or epithelial pigmentation (Brooks et al., 2017). A variable degree of permanent fibrosis at the keratectomy site is expected in all cases (Brooks et al., 2017).

In this case series, two horses developed a secondary bacterial infection as a complication of SK, while in the literature no secondary infections following SK with conjunctival grafting (n = 5) were recorded (Gilger et al., 2005). This difference could possibly be explained by the beneficial effect of blood vessels provided by the conjunctival graft that was inserted in all cases of that study (Gilger et al., 2005). These blood vessels benefit the healing process after SK which could lead to a reduced risk of secondary bacterial infection.

When signs of infection occur, samples for bacteriologic culture should be taken immediately and antibiotic treatment should be modified accordingly. In this series, one case showed improvement after changing the topical medication based on culture and sensitivity results. In the other case, due to stromal loss related to the bacterial infection, a conjunctival pedicle graft needed to be placed to preserve corneal integrity. When melting of the corneal stroma occurs, placing a conjunctival graft over (a portion) of the defect might be indicated, depending on the severity of the infection and the reaction to topical medication (Brooks et al., 2017).

A SK can be performed safely in the standing horse using local blocks (retrobulbar, auriculopalpebral, zygomaticus and subconjunctival blocks) and stable sedation levels (Hermans et al., 2019). Nowadays, standing ophthalmic surgeries have become more and more common. An anaesthetic-related mortality of up to 0.9% has been described for elective procedures (in general) under general anaesthesia in horses (Dugdale & Obhrai, 2016; Senior, 2013). Ocular surgeries have a greater risk of unsatisfactory recoveries from anaesthesia, and a higher morbidity rate (up to 40%) than other elective surgeries (Curt et al., 2018; Parviainen, 2000). Prolonged and unsatisfactory recoveries after ophthalmic surgeries could be related to prolonged surgery time, pain from the surgery, (high volume) retrobulbar blocks and the sudden loss of vision in one eye causing disorientation (Curt et al., 2018; Parviainen, 2000). These risk factors should be considered when contemplating whether the benefit of general anaesthesia for a specific patient and procedure outweighs the risks (Senior, 2013; Vigani & Garcia-Pereira, 2014).

Long-term follow-up (up to 31 months) of the four described cases showed three horses with mild to moderate fibrosis at the area of the SK, without signs of discomfort and without recurrence of IMMK despite discontinuation of treatment. Case 3 showed recurrence of IMMK after 9 months in an area of the cornea surrounding the previously removed tissue. One superficial blood vessel marked the lateral edge of the mild central corneal fibrosis that developed at the location of the first SK. The area of the first SK did not show any signs of recurrence of IMMK. During the first surgery, all the visible abnormal tissue was removed, but this probably did not include all the affected corneal tissue. We hypothesised that the 'recurrence' of clinical IMMK in fact

represented worsening of the remaining affected corneal tissue. This case emphasises the need to remove the visibly affected area of the cornea including a proper margin to ensure all abnormal tissue is removed and to prevent recurrence.

To the authors' knowledge, this is the first case report with long-term follow-up of horses with chronic superficial and/or mid-stromal IMMK treated with a SK without application of a conjunctival graft. Benefits of a SK without a conjunctival grafting procedure are reduction of surgical procedure time, and less visual impairment created by a conjunctival pedicle graft. Results of this report show that SK can be performed in the standing sedated horse. When sufficient corneal tissue is removed (the part of the corneal surface with signs of keratitis, preferably with a proper margin from the affected region), no recurrence is to be expected in the long-term follow-up (up to 31 months).

Secondary bacterial infection is a risk that should be considered and discussed with the owner. When infection occurs, placing a conjunctival graft might be indicated, depending on the severity of the infection and the response to topical medication. More long-term follow-up studies are warranted in a larger sample population to further define the (dis)advantages, rate of recurrence and complications, of SK as a surgical treatment of IMMK.

Author's declaration of interests

No conflicts of interest have been declared.

Ethical animal research

Study involved client-owned animals. Owners gave consent for inclusion in the study.

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Authorship

E. Dieterman and H. Hermans were involved in study design. E. Dieterman, H. Hermans, I.J.M. Slenter and M.H. Boevé managed the clinical cases. N.W. Kuijpers performed the ultrasonographic examination. G. Grinwis evaluated and described the histopathologic findings. All authors were involved in preparation of the manuscript and gave their final approval of the manuscript.

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