Case Report

Clinical management of superficial complicated corneal ulcerations infected with newly identified fastidious bacteria with unknown antibiotic sensitivity in three horses

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Summary

This case series addresses a clinical approach to a specific type of infected corneal ulceration in horses; the slow-healing superficial complicated corneal ulceration involving a fastidious or possibly newly identified bacteria for which no antibiotic sensitivity can be determined. These corneal ulcerations present as superficial, nonhealing lesions that appear infected based on cellular infiltration of the cornea. Because they are superficial, they do not appear to be as serious to the referral veterinarian as deep stromal corneal ulcerations. However, superficial corneal ulcerations are complicated and slow healing, ultimately frustrating both owners and primary care clinicians. Culture results are often negative, or implicate a newly identified bacteria with no known antimicrobial sensitivity in horses.

Introduction

This case series describes three cases of superficial complicated corneal ulcerations that failed to respond adequately to a standard intensive medical treatment protocol due to infection with fastidious bacteria with no known microbial sensitivity. A fastidious bacterium is defined as an organism that has a complex nutritional requirement that allows growth only under specific nutritional conditions and are therefore difficult to culture using commonly used culture media (Andersen *et al.* 2016; Anon 2017). Fastidious bacteria are known to have a slow growth rate and therefore often have a less rapid clinical progression vs. nonfastidious bacteria that are frequently very proliferative (Andersen *et al.* 2016; Anon 2017). Nonfastidious bacterial pathogens described in the equine cornea include beta-haemolytic *Streptococcus* and *Pseudomonas aeruginosa*, both having the capability of rapid corneal infiltration with devastating consequences including keratomalacia (melting corneal ulcerations) and corneal rupture (Brooks *et al.* 2000; Clode 2011). Fastidious bacteria can be responsible for 'no growth' or 'unknown bacterial growth' results encountered in some corneal ulceration bacterial culture results. Performing both culture/sensitivity and cytology with Gram stain from these lesions is important to maximise the sensitivity and specificity of the diagnostic evaluation (Clode 2011; Featherstone and Heinich 2013). Cytology can identify the ulcer as infected and the Gram stain will assist with antibiotic selection for treatment in cases that fail to culture organisms. DNA sequencing is another tool that can be used to identify fastidious bacteria; however, direct sensitivity testing cannot be performed using this technology and a literature search is necessary to try to ascertain this information (Woo *et al.* 2008; Alves Valones *et al.* 2009).

The aim of the present article is to report three equine cases with superficial complicated corneal ulcerations and present a review of recommended routine diagnostic tests and a standard intensive medical treatment protocol. A plan for additional diagnostic testing and medical therapy, in case the routine diagnostic testing and standard intensive medical treatment protocol are unsuccessful, is also reviewed.

Case series

Three horses which presented to the University of Minnesota Piper Equine Hospital (UMN PEH) for superficial corneal ulcerations nonresponsive to medical treatment in primary care settings are reviewed. All had evidence of superficial complicated corneal ulcerations with corneal cellular infiltration and anterior uveitis. In each case, diagnostic testing of the ulcerations was positive for fastidious bacteria, either only identified cytologically, or with nonspecific culture results limited to identification of the growth of uncharacterised organisms. Cytology from the corneal ulcerations was evaluated by board-certified clinical pathologists from UMN Veterinary Medical Center (UMN VMC). To optimise the potential for recovery of bacteria, culture swabs were directly plated using four different culture plates (Chocolate, Blood, Columbia CNA and MacConkey culture agars) and submitted to a specialised veterinary reference laboratory[1]. The culture plates were incubated in a CO_2 environment at 35°C and checked every 24 h for growth. Standard bacteriology and microbiology tests such as phenotypic growth on blood agar and chocolate, catalase testing, Gram stain and MALDI-TOF sequencing[2] were performed to identify the specific bacteria. DNA sequence analysis was used in one case (Case 1) in which standard bacteriology tests failed to identity the organism. This DNA sequence analysis was performed using a 16S DNA gene amplification and Sanger sequencing analysis. The DNA sequence analysis is a laboratory developed test[1] for

TABLE 1: Standard initial intensive treatment plan for complicated infected corneal ulcerations when treated at University of Minnesota Piper Equine Hospital (UMN PEH)

	Medication	Administration route	Frequency	Dose
1	Ciprofloxacin 0.3% ophthalmic solution[1]	Topical	q. 2 h	0.2 ml
2	Cefazolin 50 mg/ml ophthalmic solution[2]	Topical	q. 2 h	0.2 ml
3	Voriconazole 1% ophthalmic solution[2]	Topical	q. 4 h	0.2 ml
4	Autoloug serum[3]	Topical	q. 1 h	0.2 ml
5	EDTA 0.2% ophthalmic solution[4]	Topical	q. 1 h	0.2 ml
6	Atropine 1% ophthalmic solution[5]	Topical	q. 6 h	0.2 ml
7	Muro128 ophthalmic solution (5% NaCL)[5]	Topical	q. 6 h	0.2 ml
8	Flunixin meglumine 50 mg injectable solution[6,*]	i.v./per os	q. 12 h	Initial dose 1.1 mg/ml for 3 days
	Flunixin meglumine 50 mg injectable solution[6,*]			Decreased to 0.8 mg/kg for 3 days
	Flunixin meglumine 50 mg injectable solution[6,*]			Decreased to 0.5 mg/kg long-term
9	Gastrogard 6 g oral paste[7]	per os	q. 24 h	2 mg/kg

[1]Falcon Pharmaceuticals, Ltd, Fort Worth, Texas, USA; [2]Compounded from UMN PEH's pharmacy, Falcon Heights, Minnesota, USA; [3]Collected from the patient; [4]Compounded from UMN PEH's pharmacy; [5]Bausch and Lomb, Rochester, New York, USA; [6]Merck Animal Health, Madison, New Jersey, USA; [7]Merial, Plymouth, Minnesota, USA.
*PCT and TP would be monitored periodically when treated with this medication.

bacterial identification using an ABI 3130× capillary sequencer.

All three horses were initially treated with a standard intensive medical treatment protocol that failed to resolve ulcerations and cellular infiltration (**Table 1**). No clinical response was noted until an antibiotic therapy specifically selected to target fastidious bacteria was added to the treatment plan. All lesions healed successfully, demonstrating the value of cytology, Gram stain, culture, and for one case, DNA sequencing, for evaluation of superficial complicated corneal ulceration in horses. Utilising multiple diagnostic modalities optimises the refinement of treatment protocols to improve the prognosis for these equine patients.

Case 1

A 17-year-old American Paint Horse mare presented to UMN PEH for evaluation of nonhealing corneal ulceration in the left eye (OS). The referring veterinarian had removed a foreign body from the cornea 4 days prior to presentation, placed a subpalpebral lavage system and started treatment with topical ofloxacin (0.3% ophthalmic solution)[3], cefazolin (compounded 50 mg/ml ophthalmic solution)[4], autologous serum, atropine (1% ophthalmic solution)[5] and topical silver sulfadiazine cream (1% Silvadene cream)[6] every 6 h, as well as systemic flunixin meglumine (Banamine 50 mg/ml, 1.1 mg/kg bwt)[7] twice daily. There was minimal clinical response and the referral veterinarian was concerned about depth of the ulcer. On presentation, there was a 12 × 12 mm large superficial complicated corneal ulcer infiltrated with white blood cells OS consistent with a possible infection (**Fig 1**). The cornea was diffusely oedematous and vascularised from the dorsal and ventral aspects of the limbus, but the vascularisation did not reach the ulcerated area. The central aspect of the corneal ulcer had developed a 5 × 5 mm large bulla (hydrops) causing keratoconus formation. Moderate to severe anterior uveitis was present with +3 flare, fibrin and a midsized pupil. Cytology and culture/sensitivity was submitted from the corneal ulceration and the horse hospitalised on an intensive topical treatment protocol

(**Table 1**). The large bullae responded well to hypertonic saline treatment within a few days. The corneal ulceration did not demonstrate melting or stromal loss; however, only minimal healing occurred over the subsequent 2 weeks during hospitalisation for the intensive topical medication protocol (**Table 1**). Cytology of the corneal ulceration revealed moderate corneal epithelial atypia and no infectious agents (**Fig 2**). Culture results demonstrated scant growth of Gram-positive cocci requiring DNA sequencing for identification. The owner elected to have this performed despite the lack of sensitivity information. The DNA sequence results were available 14 days later and revealed *Macrococcus carouselicus*, a novel Gram-positive bacteria not previously identified in equine corneal ulcerations. According to the literature, *Macrococcus carouselicus* can harbour a methicillin resistance gene, potentially leading to the poor response to topical antibiotic treatment with ofloxacin and cefazolin (Kloos *et al.* 1998; Rubin and Chirino-Trejo 2010; Hiramatsu *et al.* 2013; Anon 2016). Based on this information, chloramphenicol (compounded 1% ophthalmic solution)[4] was added to the treatment protocol every 4 h (Fukuda *et al.* 2002). The horse was discharged to the referral veterinarian on chloramphenicol and by decreasing the frequency of treatments in the treatment plan in **Table 1**. The referral veterinarian reported that the corneal ulceration healed within 5 days after starting treatment with chloramphenicol. Only corneal fibrosis (corneal scar) remained at the 14 day recheck appointment at UMN PEH (**Fig 3**). Approximately 20 days after presentation to UMN PEH, the referral veterinarian reported the corneal ulceration to be healed. Thus, resolution of the ulcer was relatively rapid once appropriate antimicrobial therapy was instituted.

Case 2

A 5-month-old Arabian colt presented to UMN PEH for evaluation of a complicated corneal ulceration in the right eye (OD). The colt was found on pasture with a severely painful eye and the owner was concerned that the eye could have been
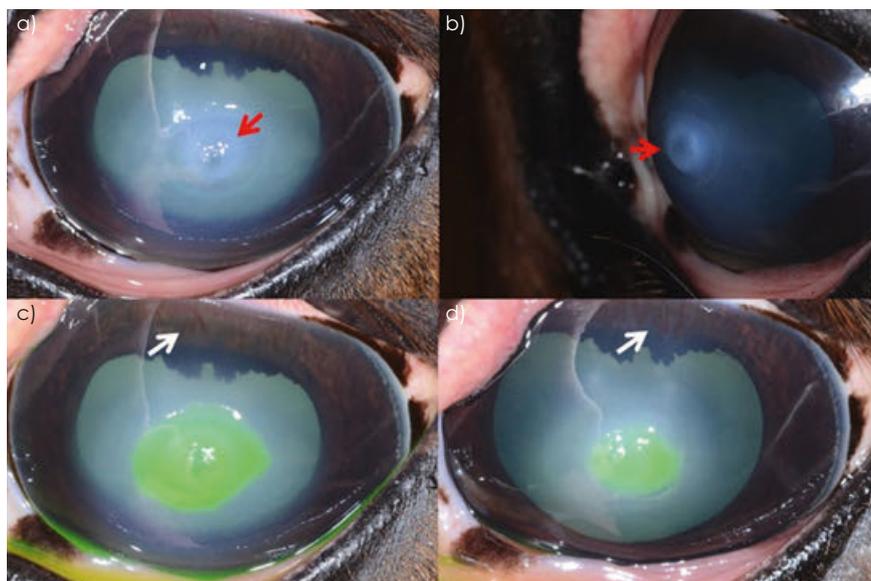
Fig 1: *Case 1. a) A superficial complicated corneal ulceration was present in the central aspect of the cornea with cellular infiltration, indicating an infection/inflammatory process (red arrow). b) The corneal ulceration had a large corneal bulla in the central aspect of the ulcer. This bulla caused the cornea to have keratoconus formation (red arrow). c) Fluorescein stain of the corneal ulceration revealed a larger superficial corneal ulceration on the day of presentation. Only a mild vascular response was infiltrating the cornea from the dorsal and ventral aspect (white arrow). d) The corneal ulceration only showed very mild signs of healing compared with the ulceration size at presentation after 14 days on intensive topical medication (Fig 1c). Also the vascular response in the cornea was very mild (white arrow).*
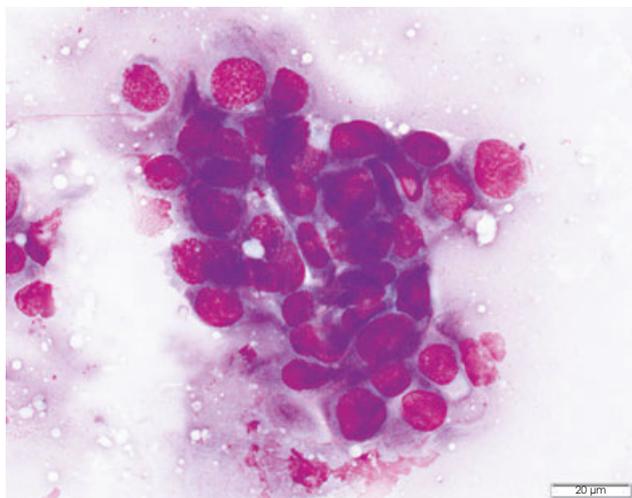

Fig 2: *Inflammation and infectious organism are not identified in this corneal cytology swab. Moderate corneal epithelial atypia was present and evaluated to be a part of the corneal healing process (50× Wright Giemsa).*

Fig 3: *Corneal ulceration in Figure 1 after healing. The area has corneal fibrosis (corneal scar), but no fluorescein stain uptake.*

injured during a storm the previous day. A picture of the eye was sent via text to the regular veterinarian by the owner and the veterinarian recommended seeing an ophthalmologist as soon as possible. On presentation, OD was diagnosed with a 6 × 6 mm large superficial complicated corneal ulceration with severe white blood cell infiltration indicative of a possible infection (**Fig 4**). The cornea had moderate to severe diffuse corneal oedema and 1–2 mm short blood vessels were infiltrating the cornea from the medial limbus. The anterior chamber w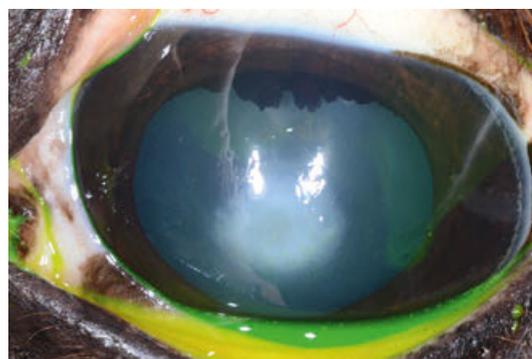as difficult to examine due to corneal opacity, but fibrin and a miotic pupil could be appreciated consistent with severe anterior uveitis. A subpalpebral lavage system[8] was placed and the colt hospitalised on intensive topical medication (**Table 1**). Cytology and culture/sensitivity was submitted from the corneal ulcer. Cytology revealed septic suppurative keratitis with many neutrophils and epithelial cells. Extracellular medium sized rod-shaped bacteria were noted adhered to the epithelial cells (**Fig 5**). The colt developed a large hydrops in the cornea 3 days after presentation and a tarsorrhaphy was placed to put pressure on the oedematous cornea. The corneal ulceration and pupil could still be evaluated at the medial canthus after the tarsorrhaphy procedure and the pupil became more dilated over the following 2 days, indicating better control of the anterior uveitis. The ulceration did not improve with initial therapy while awaiting culture results. Culture demonstrated scant growth of *Abiotrophia/Granulicatella* spp. and the laboratory would not
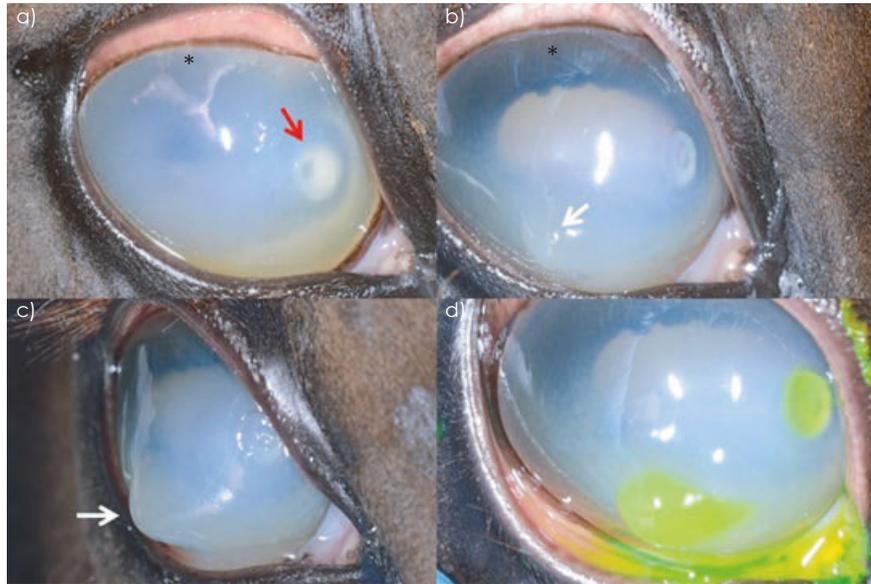
Fig 4: Case 2. a) The colt presented with superficial complicated corneal ulceration with cellular infiltration in the medial aspect of the cornea (red arrow). The cornea had diffuse moderate to severe corneal oedema and only mild corneal vascularisation was present (black asterisk indicates only mild corneal vascularisation). The colt also had severe secondary uveitis with fibrin, +3–4 flare and a miotic pupil. b) Over the next couple of days, the secondary uveitis responded to medical treatment, but the cornea developed corneal hydrops (white arrow). The corneal ulceration and vascularisation did not show sign of healing (black asterisk indicates only mild corneal vascularisation was still present). c) Cranial-caudal view of corneal hydrops (white arrow). d) The corneal hydrops developed a superficial corneal ulceration due to exposure from lagophthalmia. A fluorescein stain of the cornea revealed no signs of healing of the corneal ulceration 3 days after presentation.
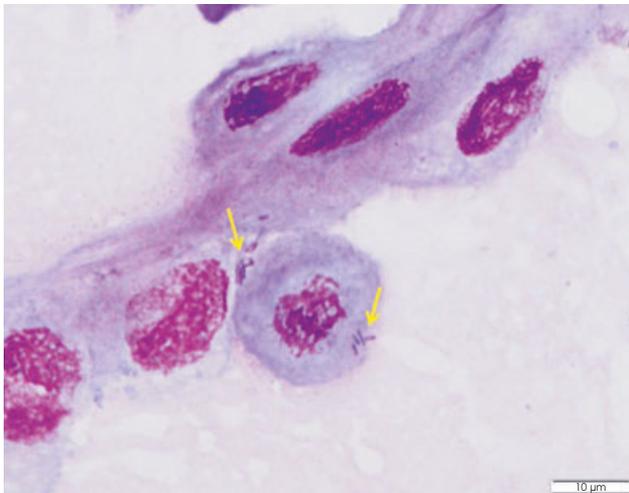

Fig 5: Septic suppurative inflammation was diagnosed on this corneal cytology swab from Case 2. Extracellular medium rods were noted and commonly found to be adhered to corneal epithelial cells (yellow arrows; 100× Wright Giemsa).

give sensitivity for these bacteria due to lack of information for sensitivity in horses. A literature search on *Abiotrophia/Granulicatella* spp. indicated these organisms were originally known as nutritionally variant streptococci, a component of normal human oral and intestinal flora (Zheng *et al.* 2004). They are fastidious bacteria and thus it is relatively uncommon to recover them from clinical specimens, but they have been reported to cause otitis media, brain abscess and septic arthritis in man (Zheng *et al.* 2004). Resistance to many of the common

ophthalmic antibiotics has been previously reported in the literature and owner financial restrictions required selection of the least expensive effective antibiotic. One paper described good efficacy of gentamicin in combination with penicillin in man with endocarditis caused by *Abiotrophia/Granulicatella* spp. (Zheng *et al.* 2004; Adam *et al.* 2015). Topical gentamicin (Gentamicin sulfate 0.3% ophthalmic solution)[9] q. 2 h was therefore added to the topical treatment plan and this would potentially also be effective against the extracellular rod bacteria identified cytologically. The colt was discharged to a lay-up stable facility and returned for a recheck 10 days later. The tarsorrhaphy was removed and the corneal ulceration was almost healed with only a small (1 × 1 mm) area of fluorescein positivity. The hydrops had healed with corneal fibrosis and the pupil was mydriatic in response to atropine treatment. The anterior chamber had no signs of active anterior uveitis (**Fig 6**). The colt returned to the lay-up stable with a plan for decreasing the medical treatment protocol over 2 weeks. At the last recheck, the corneal ulceration had healed completely with scar formation. The anterior uveitis was still resolved, but the colt showed signs of a cataract that could have been pre-existing, might have been caused by trauma, or have been due to the severe secondary anterior uveitis (**Fig 7**). It took 31 days from when the colt was presented to UMN PEH for the corneal ulceration to heal.

Case 3

A 12-year-old Thoroughbred gelding presented to UMN PEH for evaluation of a nonhealing corneal ulceration of the left eye (OS). The referral veterinarian had treated the gelding with topical triple antibiotic and autologous serum for 3 weeks.
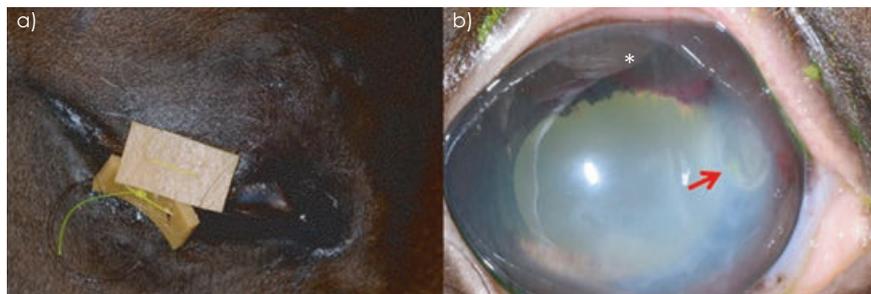
Fig 6: Case 2. a) A tarsorrhaphy was placed due to corneal hydrops. Gentamicin ophthalmic solution was also added to the intensive topical medical treatment. b) The tarsorrhaphy was removed 1 week after it was placed and the hydrops had healed with scar tissue. The corneal ulceration was almost healed and only a small area took up fluorescein stain (red arrow). The vascular response in the cornea was still only mild, but had reached the corneal lesion (white asterisk).
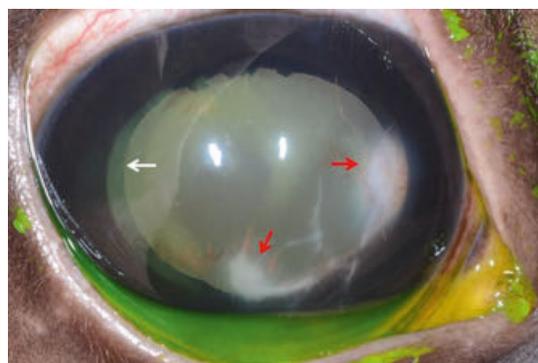

Fig 7: Case 2. The corneal ulceration had healed completely with corneal scars 28 days after the initial presentation (red arrow). An incomplete cataract could be appreciated at this examination (white arrow). This cataract could have been present before the corneal ulceration, or could have developed due to severe anterior uveitis.

Debridement with cotton swabs had also been performed with no signs of healing. The referral veterinarian had added vaginal 2% Miconazole crème[10] to the regimen 3 days before presentation to UMN PEH; according to the owner, this may have increased ocular irritation. On presentation, a 10 × 10 mm superficial complicated corneal ulcer with severe infiltration of white blood cells forming a superficial corneal plaque was identified OS. The ventral aspect of the cornea had diffuse moderate oedema and perilimbal corneal vascularisation was infiltrating from the limbus 5–7 mm into the cornea, but stopped short of the ulcer (**Fig 8**). Severe anterior uveitis was appreciated in the anterior chamber with +3 flare, a large fibrin clot behind the corneal ulcer and a miotic pupil that had started to form posterior synechiae and iris bombe at the dorsal aspect. A subpalpebral lavage system[9] was placed and the gelding hospitalised on intensive topical medication (**Table 1**). Cytology and culture/sensitivity was submitted from the corneal ulcer.

Cytology result revealed septic suppurative keratitis with moderate numbers of neutrophils and predominately extracellular bacteria with no signs of fungal hyphae. Gram stain confirmed these bacteria to be Gram-positive cocci (**Fig 9**). Culture result was negative.

The horse was hospitalised for 5 days, during which time the secondary anterior uveitis improved with dilation of the pupil and resolution of the fibrin. The corneal ulceration did not respond to therapy. Surgical options including superficial keratectomy and conjunctival graft were discussed with the owner, who elected to continue medical treatment at home at a lower frequency due to financial concerns. Chloramphenicol (compounded 1% ophthalmic solution)[5] was added to the treatment protocol based on the Gram stain results and lack of response to the two previous antibiotics (**Table 1**). The horse returned to UMN for a recheck 10 days after discharge and the ulcerated area had not changed in size, depth or appearance, except for the ventral aspect of the ulcer, where granulation tissue had formed. At a subsequent recheck appointment at UMN 10 days after the first, the corneal ulcer was almost completely granulated and only a small area took up fluorescein stain. The referral veterinarian rechecked the eye again 10 days later and the area was completely healed with granulation tissue and no signs of fluorescein stain uptake (**Fig 10**). It took 35 days from presentation to UMN PEH until the corneal ulceration had healed.

Discussion

This case series describes three cases of superficial complicated corneal ulceration that failed to respond adequately to a routine intensive topical medication protocol. Fastidious bacteria, not previously implicated in equine corneal ulceration, were identified in two of the three cases and cytology revealed a bacterial infection, but culture was negative in the last case. DNA sequencing was used in one case to identify the fastidious bacteria. These three cases demonstrate the frustration of poorly responsive ocular lesions and some options for clinical resolution. A 'take home message' from this case series is to be consistent in diagnostic testing/data collection practices. Always perform both cytology and culture/sensitivity since these modalities are complementary and used together are more likely to successfully identify pathogens. Also, DNA sequencing can be valuable if used in conjunction with the medical literature on susceptibility patterns to optimise antimicrobial selection. Also, it is important to use a laboratory capable of working with fastidious organisms. The University of Minnesota ophthalmology service performs direct plating of specimens on four different culture plates (Chocolate, Blood, Columbia CNA and MacConkey culture agars) to maximise organism recovery based on the recommendations of the supporting laboratory[1]. Despite this approach, there are still cases where
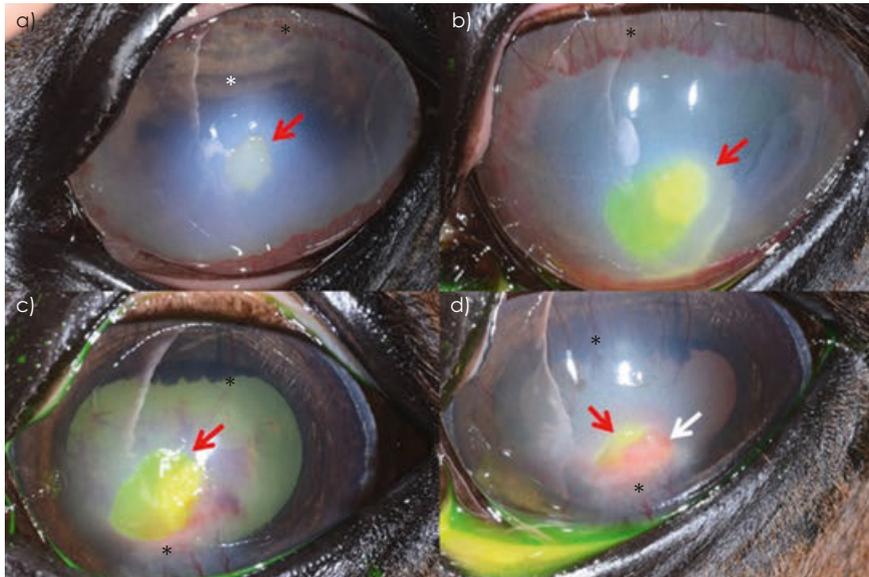
Fig 8: Case 3. a) The horse presented with a superficial complicated corneal ulceration that had severe cellular infiltration (red arrow). Only mild vascular response could be appreciated in the cornea (black asterisk). Severe anterior uveitis was present with pupillary occlusion and starting iris bombe (white asterisk), b) One week after the horse was started on intensive medical treatment. The corneal ulceration is still present with no signs of healing (red arrow). The corneal vascularisation is slowly infiltrating the cornea (black asterisk), c) Three weeks after the initial presentation. The corneal ulceration is healing with granulation tissue due to corneal vascularisation that has reached the ventral, but not yet dorsal aspect of the ulcer (black asterisks). d) Five weeks after the initial presentation. Three-quarters of the corneal ulceration has granulated (white arrow) due to the corneal vascularisation that has reached the ulcer (black asterisks). There is still one small area of fluorescein stain uptake indicating that the ulcer has not yet healed (red arrow).
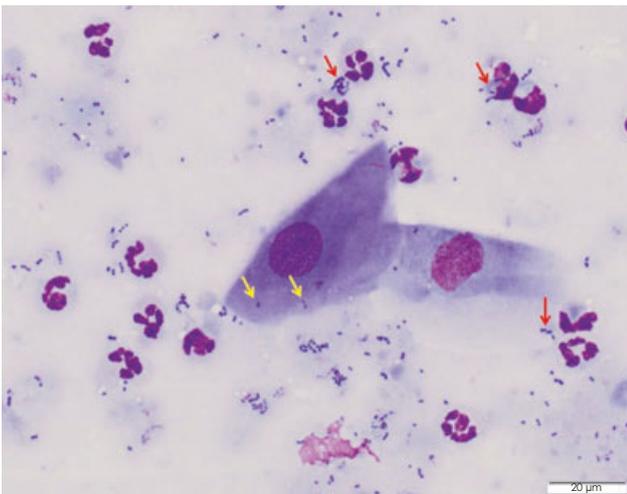

Fig 9: Septic suppurative inflammation was diagnosed on this corneal cytology swab from Case 3. Typical Gram-positive cocci in chains were noted to be present and in some areas the cocci seemed elongated and almost rod-shaped (red arrows). The coccid were predominately extracellular (red arrows), but also present intracellular (yellow arrows; 50× Wright Giemsa).

Fig 10: Case 3: 7 weeks after the initial presentation. The corneal ulceration has healed with granulation tissue. This area will remodel over the next few months and the horse will have a corneal scar in the area of the granulation tissue.

cytology will identify bacteria that cannot be successfully grown on culture. Repeat collection of bacterial culture samples is an option for poorly responsive lesions; however, this represents an extra expense for the owner and may fail to yield positive results. Therefore the cytology result and Gram stain

are valuable to evaluate the suspicion of a bacterial or fungal infection. Fungal culture is another test recommended for equine corneal ulcerations that are poorly responsive to antibacterial medications. A fungal culture result usually requires between 3 and 4 weeks (Featherstone and Heinich 2013), so it is not advisable to await the results of culture prior to treatment in suspected fungal keratitis (Brooks 2008; Clode 2011). This is a significant disadvantage of using fungal culture as a primary screening test for fungal infections. Submission of cytology slides to a laboratory for examination by a board-

certified veterinary clinical pathologist can be a much faster way of confirming a fungal infection. In cases where 'no growth' is the culture result, but a response to medical treatment is recognised, continuing the topical medication is recommended until the corneal ulceration is fluorescein negative (Clode 2011; Featherstone and Heinich 2013). Superficial complicated corneal ulcerations due to infection with a fastidious bacteria, such as the cases described here, are frustrating cases for owners and equine practitioners since they require a long time to heal using an intensive topical medication protocol. This medical protocol is expensive for the owner since these horses usually require boarding in a hospital or layup stable where their every 2–4 h medication protocol can be performed for multiple weeks. Owners of these cases are therefore interested in prognosis and outcome. This case series shows that these horses do have a good prognosis for healing with medical treatment, but resolution takes approximately 3–5 weeks and is expensive.

Conclusion

Fastidious bacteria can cause superficial complicated corneal ulceration in horses. Consistent diagnostic tests with cytology and culture/sensitivity are important to diagnose the specific bacteria in these cases and therefore be able to make the correct topical treatment plan. These cases do have a good prognosis for healing on medical treatment but they can heal slowly and can have a high economic cost for owners.

Authors' declaration of interests

No conflicts of interest have been declared.

Ethical animal research

Case report therefore no ethical approval needed.

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Authorship

M. de Linde Henriksen was the ophthalmologist who diagnosed and treated all three cases, provided clinical images and wrote the majority of the manuscript. L. Sharkey was the pathologist who read the cyotology, provided cytology images and assisted with manuscript preparation. M. Esser was the senior internist overseeing one of the cases while in hospital and contributed to manuscript editing. J. Costello was the large animal intern responsible for case management on one of the cases and also performed a DNA analysis literature search while the case was in the hospital and contributed to manuscript editing. All authors gave their final approval of the manuscript.

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[1]Marshfield Veterinary Laboratory, Waukesha, Wisconsin, USA.
[2]Bruker, Billerica, Massachusetts, USA.
[3]Falcon Pharmaceuticals, Ltd, Fort Worth, Texas, USA.
[4]Compounded from Stokes Pharmacy, Mount Laurel, New Jersey, USA.
[5]Akorn Pharmaceuticals, Lake Forest, Illinois, USA.
[6]Monarch Pharmaceuticals, Fort Worth, Texas, USA.
[7]Merck Animal Health, Madison, New Jersey, USA.
[8]Mila International Inc., Florence, Kentucky, USA.
[9]Bausch + Lomb, Rochester, New York, USA.
[10]G&W Laboratories Inc., South Plainfield, New Jersey, USA.

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