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Identification of bacteria and fungi sampled from the conjunctival surface of normal horses in South-East Queensland, Australia

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Abstract

Objective To identify bacteria and fungi found on the conjunctival surface of normal horse eyes; to investigate potential risk factors for these microflora; and to determine their susceptibility to common topical ophthalmic antimicrobials.

Animals studied A total of 95 client-owned horses were studied.

Procedures Horses within sub-tropical Australia (South-East Queensland) were sampled once between April 2012 and March 2013. A conjunctival swab was taken from each eye and cultured for aerobic bacteria and fungi. Organisms were identified by colony morphology and phenotype. Antimicrobial disk diffusion susceptibility testing for commonly used antimicrobials was performed.

Results Positive bacterial cultures were returned from 187/190 (98.4%) eyes from 94/95 (98.9%) horses. The most common species included: *Staphylococcus* spp. (25.2% of total bacterial isolates), *Bacillus cereus* (17.4%), *Bacillus* spp. (14.1%) and *Corynebacterium* spp. (8.9%). Most bacterial isolates were susceptible to neomycin and fluoroquinolones. Positive fungal cultures were returned from 111/190 (58.4%) eyes from 73 (76.8%) horses. The most common species identified included: *Penicillium* spp. (16.7% of fungal isolates), *Aspergillus* spp. (15.4%) and *Scopulariopsis* spp. (10.3%). Most ($\geq 90\%$) molds were susceptible to ketoconazole, voriconazole, itraconazole and miconazole. Yeasts were most susceptible to ketoconazole. There was no significant effect of breed, age, sex, purpose or housing of the horse or climatic conditions on bacterial or fungal culture status.

Conclusions Bacteria and fungi were commonly isolated from the eyes of healthy horses. The antibiotic and antifungal susceptibilities identified can be used as a guide for empirical therapy after cytology in the treatment of corneal ulceration in horses.

Key words: equine, conjunctiva, bacteria, fungi, antimicrobial, risk factors

INTRODUCTION

The equine eye is at risk of injury due to its large size and prominent lateral displacement, and because of the horse's temperament that predisposes it to head tossing.[1] There is also a risk of infection from exposure of the ocular surfaces to bacteria and fungi due to the environmental conditions in which the horse lives. Local innate and adaptive mechanisms protect the eye against pathogenic organisms.[2-5]

Commensal and transient bacteria and fungi can be found on the normal ocular surface and compete with pathogenic microbes for nutrients and space. In the event of ocular trauma or impairment of innate and adaptive local immune mechanisms or co-infection with

other microbes, commensal and transient microbes have the potential to become opportunistic pathogens and induce clinical ocular disease.[6]

A number of studies identifying the normal equine ocular flora confirm that gram-positive bacteria are predominant with *Staphylococcus* spp.,[7-11] *Corynebacterium* spp.,[7-9] *Bacillus* spp.,[7, 9, 10] *Streptococcus* spp.,[8, 10] *Streptomyces* spp.,[7, 9, 10] and *Micrococcus* spp.[11] identified most frequently. The most common gram-negative bacteria include: *Escherichia coli*,[8, 10] *Acinetobacter* spp.,[8, 10, 11] *Neisseria* spp.,[8, 10] *Moraxella* spp.,[8, 10] *Pseudomonas* spp.,[8, 10] and *Enterobacter* spp.[10] Age,[8, 9] sex,[8] housing,[7, 8] and geographic location[7] affected the frequency of bacterial isolation.

Over 30 genera of saprophytic filamentous molds and yeasts have been reported to cause keratomycosis in the horse.[1] These organisms are often contaminants from the local environment.[6] Fungal organisms isolated from the normal equine eye include: *Aspergillus* spp.,[8, 10-18] *Cladosporium* spp.,[8-14, 16, 18] *Penicillium* spp.,[8, 12, 13, 15-18] *Fusarium* spp.,[8, 12-14, 16] *Alternaria* spp.,[8] *Chrysosporium* spp.[9] and *Candida* spp.[14-17] Housing, [8, 12, 14, 16] breed[17] and season[19] affected the frequency of fungal isolation.

Previous studies have been conducted in the temperate parts of North America [7-10, 20] and Europe,[11-13, 15, 18] and in tropical locations such as Brazil.[12, 16] No studies have been conducted in Australia. We aimed to survey and identify the bacterial and fungal flora found on the conjunctival surface of normal equine eyes in South-East Queensland, a sub-tropical area in Australia, and to examine the risk factors of breed, age, sex, purpose and housing of the horse and climatic conditions (including season, temperature, humidity, precipitation, wind speed) on the presence of microflora. We also aimed to identify the susceptibility of the flora to common topical ophthalmic antimicrobials.

MATERIALS AND METHODS

Horses

This was a cross sectional survey based on a convenience sample of horses attending a veterinary clinic for routine procedures. Clients from three equine clinics in South-East Queensland were invited to include their horses in the study. Inclusion criteria were horses systemically normal on clinical examination by an equine veterinarian, with normal adnexa and anterior segment on examination by a specialist veterinary ophthalmologist and had not been given antimicrobials or antifungal drugs by any route during the two weeks prior to presentation.

Exclusion criteria were active inflammatory or infectious eye disease, signs of ocular pain, ocular discharge, or conjunctivitis. Quiescent healed corneal scars of greater than two years duration were not grounds for exclusion.

Each owner signed consent for participation in the study, including the informed consent for sedation and anesthesia for reasons relating to the presenting condition. The study complied with the relevant animal welfare legislation [21] and was approved by The University of Queensland Animal Ethics Committee (SVS/449/11/ACA).

Horses were sampled within 24 hours of admission to the veterinary hospital. The age, sex, breed of the horse and geographical area (postcode/zipcode) from where the horse originated was recorded. The work status (utility) and housing (paddock or stable) of each horse was noted. Weather details were obtained from the Bureau of Meteorology (<http://www.bom.gov.au>): temperature (°C) on the day of sampling, total rainfall for the day (mm), humidity (%), and wind speed (km/h) at the time of collection.

Eye examination

For horses requiring sedation, drugs were used at the discretion of the consulting clinician. Both eyes were examined in each horse. The examination was conducted indoors commencing with distant examination of globe position, size and motility and presence of blepharospasm or ocular discharge. Menace response, palpebral reflex, dazzle reflex and direct and consensual pupillary light reflexes were assessed in both eyes prior to any sedation. Close up examination of eyelids, conjunctival and corneal surfaces were performed in the dark with a Finoff transilluminator (3.5v Finoff transilluminator, Welch Allyn Australia Pty Ltd, Rydalmere, New South Wales 2116, Australia). More detailed examination with slit lamp biomicroscopy (Kowa 15, Kowa Company Ltd, Tokyo, Japan) of eyelid margins, conjunctival and corneal surfaces, anterior chamber, iris and lens was performed. The optic nerve head and retina were examined with a direct ophthalmoscope (Welch Allyn Australia Pty Ltd).

Collection of samples

Conjunctival samples were harvested without the use of topical anesthesia. A microbiology swab (Transwab^R, Sarstedt, Technology Park, South Australia, 5095, Australia) was moistened with sterile saline and then rolled gently over the exposed anterior conjunctival

surface of the third eyelid, near the medial canthus. To facilitate prolapse of the third eyelid, the eyelids were parted gently open by the sampler's thumb and index finger and the globe was retropulsed through the upper eyelid. Special care was taken to avoid touching the vibrissae, eyelids or eyelashes; if these were touched, this swab was discarded and the third eyelid was sampled again with a new moistened swab. A separate swab was used in each eye.

Each swab was then placed in the accompanying transport media (M40-A Compliant Amies medium with inorganic buffer^R, Sarstedt) and samples were kept on ice or refrigerated (4 °C) until submitted to the laboratory for culture within 24 hours.

Microbiology

Specimens were cultured for aerobic bacteria and fungi. Each swab was cultured onto blood agar (BA), MacConkey agar (MCA) and Sabouraud dextrose agar (SDA) and inoculated into 5 ml brain heart infusion broth, and incubated at 37 °C overnight prior to subculture onto BA and MCA. All media was sourced from Thermo Fisher Scientific Australia Pty Ltd, Thebarton, South Australia, 5031, Australia. The BA and MCA plates were incubated for 24-48 h at 37 °C. Bacterial cultures were reviewed at 24 and 48 h after plating. The presence or absence of bacteria was recorded. Bacteria were identified using morphology, Gram stain and standard veterinary microbiological techniques and identified to genus, and where possible, to species level.

Antimicrobial susceptibility was performed by disc diffusion as per Clinical and Laboratory Standard Institute (CLSI) guidelines for 12 antimicrobials (chloramphenicol (30 µg), ciprofloxacin (5 µg), fusidic acid (50 µg), ofloxacin (5 µg), cloxacillin (5 µg), tobramycin (10 µg), gentamicin (10 µg), neomycin (30 µg), tetracycline (30 µg), levofloxacin (5 µg), bacitracin (10 U) and cephalothin (30 µg)).[22] All antimicrobial discs were obtained from Oxoid (Thermo Fisher Scientific Australia Pty Ltd). *Escherichia coli* ATCC 25922 was the quality control organism.[22]

There are no breakpoints for topical antimicrobials therefore systemic breakpoints defined by CLSI guidelines were used where possible. [23-25] For neomycin, breakpoints supplied by the company (resistant ≤13 mm, intermediate 14-15 mm, susceptible ≥16 mm) were used for all bacteria (<http://www.oxoid.com/pdf/uk/2013-CLSI-FDA-table-update.pdf>). For *Acinetobacter* spp., chloramphenicol breakpoints recommended by Sood (2016) was used.[26] *Staphylococcus* spp. breakpoints were used with *Bacillus* spp. and *Micrococcus* spp. for all antimicrobials. If no breakpoints were available and there was no zone of

inhibition (< 7 mm), it was classed as resistant and median and range of the inhibition zone recorded.

The SDA plates were incubated at 25 °C and examined daily for up to 21 days. Subsamples of fungal specimens were stored in pure water in airtight glass bottles. Stored fungal tissue was subsampled using a sterile loop and inserted into SDA plates and incubated at 30 °C for up to 6 weeks, after which time if there was no visible growth samples were considered non-viable. Viable samples were described for top and reverse morphology, growth rate and agar discoloration. Sample identification to genera was conducted via standard lactophenol cotton blue staining and 40X light microscopy to identify reproductive structures.[27, 28] Antifungal susceptibility testing was performed by disc diffusion as per the CLSI method for antifungal disc diffusion susceptibility testing of nondermatophyte filamentous fungi or yeasts.[29, 30] *Candida krusei* ATCC 6258 was used as a quality control organism. Sensitivity to five commonly used topical antifungals voriconazole (1 ug), fluconazole (25 ug), miconazole (10 ug), ketoconazole (15 ug) and itraconazole (10 ug) was assessed. All antifungal discs were obtained from Dutech Diagnostics, Crydon, New South Wales 2132, Australia. When the optical density range for a genus was not prescribed, the optical density used for disc diffusion protocols was that of the nearest taxonomic prescribed genera. Some genera of fungi did not meet the specifications of the CLSI protocol, however where diffusion zones were clearly discernable, these results were recorded. If there was no zone of inhibition (< 7 mm), it was classed as resistant and median and range of the inhibition zone recorded.

Statistical Analysis

Bacteria were grouped into genus and Gram stain types for reporting. Selected bacteria of clinical importance are reported to species level. Summary statistics are reported as mean (standard deviation, SD) for normally distributed variables and median (interquartile range, IQR) for non-normally distributed variables, unless otherwise indicated. Categorical variables are reported as frequencies. The effects of breed, age, sex, geography and housing, temperature, rainfall, humidity and wind velocity on bacterial gram-type and fungal culture status (negative or positive) were assessed using the Chi square or Fisher's exact test. Agreement between bacterial species OS and OD of each horse was assessed using Cohen's Kappa. Data was analyzed using Stata.[31] Statistical significance was set at $P < 0.05$.

RESULTS

Horse characteristics

Ninety-five client-owned horses were presented over a 12-month period from April 2012 until March 2013, thus a total of 190 eye swabs were investigated. Median age was 7.0 years, IQR 4.5-13 years, range 2 months to 27 years. There were 7 entire males, 42 geldings and 46 entire females of which 2 were pregnant. The breeds included 84 (88.4%) purebreds and 11 (11.6%) crossbreds.

Eighty-seven of 95 horses (91.6%) were housed outdoors in grassed paddocks. Seven horses were stabled and one horse had access to stable and paddock. Fifty-five horses presented either as couples ($n = 18$), trio ($n = 9$) or quad ($n = 8$), or as a group of 20 horses: the horses from each group came from the same premises. Seventy-five horses (78.9%) were used for recreational riding and 20 horses (21.1%) for breeding.

In 18 (18.9%) horses, collection of samples was taken under sedation with xylazine, detomidine or butorphanol for procedures unrelated to this study. Examination of the anterior segment revealed five horses with unilateral corneal scars and one horse with bilateral corneal scars, all had been present for over two years.

Geography and climate

Most horses were sampled from four main locations west of Brisbane, within an approximate radius of 100 km from the center of the collection zone. Collections commenced in late autumn/fall in Australia with a total of 14 horses swabbed in autumn (5 in April, 9 in May); 23 in winter (3 in June, 18 in July, 2 in August), 15 in spring (1 in September, 7 in October, 7 in November), and 34 in summer (6 in December, 16 in January, 12 in February) and concluded in early autumn/fall (9 in March).

Mean seasonal temperature ranged from 15.0 to 27.0 °C, mean wind velocity from 0 to 31 km/h, mean humidity from 43 - 98%, and rainfall from 0 - 10.4 mm/day (Fig. 1). Gram-positive cocci were most commonly isolated, followed by gram-positive rods, gram-negative bacteria and gram-positive bacilli (Fig 2). There was no association between bacterial gram-type and the distribution of age ($P = 0.80$), sex ($P = 0.66$), season ($P = 0.24$), temperature ($P = 0.33$), wind velocity ($P = 0.33$), or humidity ($P = 0.14$). There was also no association between fungal culture (positive or negative) and the distribution of age ($P = 0.06$), sex ($P = 0.68$), season ($P = 0.99$), temperature ($P = 0.26$), wind velocity ($P = 0.14$), or humidity ($P = 0.33$).

Bacterial Isolates

Four-hundred and sixty bacterial isolates from 31 genera were identified from 187/190 (98.4%) eyes of 94/95 (98.9%) horses (Table 1). Of these gram-positive bacilli/coccobacilli, (41.3%) and gram-positive cocci, (39.1%) were the predominant bacterial species identified, followed by gram-negative bacilli (18.9%) with gram-negative cocci (0.6%) found rarely. One horse had a positive culture from only one (OS) eye, and one horse had no positive culture from either eye.

The median (range) for the number of positive cultures per horse was 5 (0 - 10). Median (range) for OS and OD was 2 (0 - 6). Seventy-two horses (75.8%) had the same bacteria cultured from both eyes. Forty-one horses had one bacterial type in common with both eyes, 27 had two in common and 4 had 3 in common. Twenty-two horses (23.2%) had no bacteria in common between OD and OS.

Of the 460 bacterial isolates, 107 were from 'pairs' from horses which had the same microbe in both eyes, thus 46.6% (214/460) of positive cultures were associated with bilateral colonization. The remaining 246 (53.5%) bacterial isolates were derived from horses which did not have the same bacterium in the corresponding opposite eye.

For horses where the same bacterial isolate was cultured from OD and OS, the most common organisms were *Staphylococcus* spp. (43 pairs), *Bacillus cereus* (22 pairs) and *Bacillus* spp. (14 pairs). Other bacteria recovered from both eyes in the same individual were *Micrococcus* spp. (9 pairs), *Corynebacterium* spp. (9 pairs), *Streptococcus* spp. (2 pairs) and Gram-negative bacilli (2 pairs). *Enterobacter* spp., coagulase negative *Staphylococcus* spp., *Pseudomonas* spp., *Moraxella* spp., *Escherichia* spp. and *Acinetobacter* spp. were cultured from 1 pair of eyes each.

Kappa tests for agreement were performed for bacteria that had 9 or more pairs (from both eyes). Agreement was less than expected for all types, with the kappa statistic being negative and *P* value > 0.90 in every case. This indicates that we found no evidence to suggest that bacterial colonization with a particular organism in one eye increases the probability that the same organism will colonize the other eye.

Gram-positive cocci had high susceptibility (> 90%) to bacitracin, chloramphenicol, ciprofloxacin, fusidic acid, levofloxacin, neomycin and ofloxacin (Table 2a); gram-positive bacilli to chloramphenicol, ciprofloxacin, fusidic acid, gentamicin, levofloxacin, neomycin, ofloxacin and tobramycin (Table 2b); and gram-negative bacilli to ciprofloxacin, gentamicin, levofloxacin, neomycin, ofloxacin, tetracycline and tobramycin (Table 2c).

Fungal isolates

One hundred and eleven of 190 (58.4%) eyes from 73/95 (76.8%) horses were fungal culture positive (Table 3). Seventy-eight samples (78/111, 70.3%) from 60 horses, were identified and classified as either molds ($n = 67$, 85.9%) or yeasts ($n = 11$, 14.1%). Thirty-three cultures from 13 horses were not viable on re-culturing and were not identified. The median (range) number of fungi cultured and identified from an eye was 1 (1 - 5).

Fungi were isolated from both eyes in 38 horses, from the right eye only in 14 horses and from the left eye only in 21 horses. No fungi were grown from either eye in 22 horses. Only two horses had the same mold in both left and right eyes (one pair of *Scopulariopsis* spp. and one pair of *Fusarium* spp.). No horse had the same yeast in both left and right eye.

Of the 78 positive fungal isolates, 4 (5.1%) were from 'pairs' or from horses that had the same genera in both eyes. Therefore, 94.9% of positive fungal cultures were associated with unilateral infection with respect to genus. Nineteen fungal cultures occurred in the context of infection in both eyes in eight horses with a different genus in each eye.

Filamentous molds were highly susceptible ($> 90\%$) to itraconazole, ketoconazole, miconazole and voriconazole (Table 4a); and yeasts were highly susceptible (100%) to ketoconazole, but less susceptible (82%) to voriconazole (Table 4b).

Thirty-eight horses (38/95, 40%) had a positive bacterial and fungal culture in both eyes. Thirty-four (34/95, 35.8%) had a positive bacterial culture in both eyes and positive fungal culture in either the left or right eye. Twenty-one horses (21/95, 22.1%) had bacteria cultured from both eyes, but no fungi cultured from either eye. Of the remaining two horses, one had no bacteria but fungi cultured from the right eye, and the other had bacteria but no fungi cultured from the right eye.

In the five horses that had unilateral old corneal scars, there were 14 bacteria cultured from scarred eyes and 13 bacteria cultured from non-scarred eyes. There was no difference in the number of bacteria cultured in eyes between those with and without scars (Fisher's exact test, $P = 0.96$).

The distribution of gram-types from the co-housed group of horses ('clustered horses') was compared to a reference distribution of gram types which was estimated from 205 isolates from 40 horses that were housed individually. The comparison was restricted to clusters where there were more than 7 isolates in the cluster. Gram-negative cocci were excluded from the comparison because there were too few isolates (3 isolates from 2 horses). In the reference group 45/205 (21.9%) of isolates were gram negative bacilli, 68/205 (33.2%) gram positive bacilli/coccobacilli, and 92/205 (44.9%) gram positive cocci. The largest group of co-housed horses ($n = 20$ horses with 94 isolates) had a higher proportion of gram-positive

301 bacilli (53.3% vs. 33.2%, $P < 0.001$) and a lower proportion of gram-positive cocci (29.3%
302 vs. 44.9%, $P = 0.002$) than the reference group. There was no difference in the proportion of
303 gram-negative bacilli (17.4% vs. 21.9%, $P = 0.25$). There were no differences in the
304 distribution of gram types between any other cluster of horses and the reference group ($P >$
305 0.05 for all comparisons). Eight horses were stabled and 87 housed outdoors. Of 190 eyes,
306 12/16 (75.0%) from the 8 stabled horses harboured fungi, compared with 99/174 (56.9%) of
307 eyes from outdoor-housed horses ($P=0.23$).

308 DISCUSSION

309 Infectious keratitis is a common, painful and potentially blinding disease in the horse. The
310 pathogenesis suggests that normal ocular microflora may become opportunistic pathogens
311 and induce clinical disease if the epithelium is traumatized or co-infected with other
312 organisms.[3, 6] Our observations of the predominance of gram-positive cocci and bacilli in
313 normal flora and the high likelihood they are susceptible to commonly available first line
314 topical antimicrobials, such as chloramphenicol, fusidic acid and gentamicin, will help equine
315 veterinarians treat these corneal diseases.

316 The predominant gram-positive and gram-negative bacteria identified in this study are
317 comparable to those found in the USA [7, 8, 10, 19] and in the UK [11] although fewer *E.*
318 *coli*, *Moraxella* spp. and *Neisseria* spp. were found in our study.

319 The predominant molds identified were similar to studies in North America,[8, 10, 19,
320 20] Europe [11, 13-15, 17, 18] and Brazil,[12, 16] although in our study *Aspergillus* spp.
321 were three times more commonly isolated than *Fusarium* spp.

322 We did not identify any association between bacterial and fungal colonization. Of the
323 190 eyes we examined 98.4% cultured bacteria and only 58.4% cultured fungi. This contrasts
324 with Moore *et al.* (1988) where similar percentages of bacteria (50% of eyes) and fungi
325 (42.5% of eyes) were found, and that bacteria nearly always co-existed with fungi.[8] This
326 study was conducted in hospitalized and stabled horses, which might explain the higher
327 prevalence of co-harbored bacteria and fungi. A similar interaction between fungi and
328 bacteria in eyes has been reported from horses housed in open spaces, although data was not
329 expressed as percentages of bacteria and fungi from eyes.[9] Another study demonstrated
330 90% eyes cultured bacteria and 78% eyes cultured fungi, but the interaction between these
331 organisms were not described. [10]

332 Our finding of no difference in the number and type of bacterial or fungal isolates
333 between scarred and non-scarred eyes suggests that old corneal scars do not affect the type of
334 commensal bacteria and transient fungi on the ocular surfaces.

The risk factors of age and sex of the horse in our study had no effect on the frequency or type of organism isolated. Other studies have shown that young adult male horses had an increased frequency of bacteria cultured [8] and younger horses had an increased frequency of gram-negative rods and fungi.[9] In our study there was no association between breed and frequency and type of isolates, although a previous study showed that ponies had a higher frequency of fungal infection compared with larger breeds.[17]

It is uncertain as to why the larger group of horses that co-housed (n=13) had a higher frequency of gram-positive bacilli than other groups of horses, other than more bacilli were present within that environment of mares used for reproductive purposes.

The effect of climate (subtropical), season (autumn to autumn) and weather (temperature, humidity, rainfall, wind speed and velocity) had no difference in frequency and type of bacterial and fungal isolates in our study. The absence of extreme differences in weather patterns throughout the year, may explain why the type of transient and commensal ocular bacteria and fungi remained constant, similar to studies that showed no difference in the frequency of fungal isolation between seasons in different climates.[8, 9, 11]

Antibiotic susceptibility testing of bacterial isolates in earlier studies gave high susceptibility to chloramphenicol,[8, 10] gentamicin,[8, 11] tetracycline,[8, 11] neomycin[8] and polymixin B,[8] but a low susceptibility for fusidic acid.[11] Our study also showed high susceptibility to fluoroquinolones. Testing of fungal isolates in these earlier studies had high susceptibility to natamycin, nystatin, and miconazole, but low susceptibility to fluconazole and ketoconazole, [8] while a recent study demonstrated that most fungi were susceptible to voriconazole and itraconazole, but more resistant to fluconazole.[18] Our study also supports that both filamentous molds and yeasts were more resistant to fluconazole.

We conducted a convenience survey rather than a formal random sample of the population of horses, so there may be some selection bias. Nevertheless, because subjects were clinically healthy horses presenting for routine procedures, they are likely to be representative of horses in the study catchment. Increased yield of fungal and bacterial isolates may have occurred if the samples were inoculated directly into culture broths at sampling. Such a technique may have overcome problems with stored samples for identification and susceptibility testing. The lack of clinical breakpoints for all topical antimicrobials and lack of breakpoints for some bacteria and fungi against certain antimicrobials makes it difficult to interpret susceptibility patterns. However, criteria and protocols were standardized where these breakpoints were lacking. Future studies may

benefit from molecular identification of bacteria and fungi [32] and sampling eyes from clinical cases of infectious keratitis.

Best practice therapy for acute equine corneal ulceration would include first obtaining swabs for culture and cytology and corneal scrapes for cytology. As each eye harbors different microbes, it is recommended each diseased eye be sampled. Initial antimicrobial therapy could be based on identification of gram status of any bacteria, and presence of, filamentous molds and yeasts on cytology. Our findings suggest that commonly available first line topical antimicrobials chloramphenicol, fusidic acid, neomycin and fluoroquinolones would be suitable for gram-positive cocci and bacilli; gentamicin, neomycin, tobramycin and fluoroquinolones would be suitable for gram-negative bacilli. Where fungi are identified, suitable first-line therapy would be ketoconazole, miconazole and voriconazole for filamentous molds; and ketoconazole for yeasts. Dual therapy with an antibiotic and antifungal drug may be recommended if cytology were not available and where fungal keratitis is known to be more commonly diagnosed in certain geographic areas. Changes to antimicrobial therapy may be required following formal culture and susceptibility testing.

A better understanding of the ocular flora in normal horses' eyes will assist veterinarians in making best practice selection of topical antimicrobial drugs when treating eye trauma and infections. Early cytological investigation of bacteria and fungi and identification of their antibiotic and antifungal sensitivities should be used as a guide for empirical therapy. It is hoped that such evidence-based medicine will reduce the incidence and severity of infectious corneal disease in the horse.

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FIGURE LEGENDS

Figure 1. Weather patterns over the study period from Autumn 2012 until Autumn 2013. Temperature ($^{\circ}$ Celsius), Rainfall (mm), Humidity (%) and Wind speed (km/hr) were plotted with respect to seasons (Autumn, Winter, Spring and Summer 2012 and Autumn 2013). Note that humidity was highest during autumn and winter months, although traditionally humidity is highest during summer and early autumn in South-East Queensland.

Figure 2 . Sum of bacteria type (based on Gram stain classification and morphology) with respect to date of study. Bacteria type was classified as gram-positive cocci, gram-positive bacilli, gram-negative bacilli and gram-negative cocci. The proportion of bacteria (0-50) was plotted with respect to seasons (Autumn, Winter, Spring and Summer 2012 and Autumn 2013). There was no significant effect of season, climatic conditions and weather patterns on bacterial or fungal culture status.

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Table 1. Bacterial types and species reported as number of isolates OD, OS and OU for 187 eyes from 94 horses*. A total of 460 bacterial isolates were identified as either gram-positive cocci (180) or bacilli/coccobacilli (190) or gram-negative bacilli (87) or cocci (3).

Gram-positive cocci	Number OD/OS (OU)	Gram-positive bacilli/coccobacilli	Number OD/OS (OU)	Gram-negative bacilli	Number OD/OS (OU)	Gram-negative cocci	Number OD/OS (OU)
Staphylococcus spp.	54/62 (116)	Bacillus cereus	39/41 (80)	Enterobacter spp.	7/8 (15)	Moraxella bovoculi	1/1 (2)
Micrococcus spp.	19/13 (32)	Bacillus spp.	34/31 (65)	Acinetobacter spp.	5/7 (12)	Neisseria sp.	1/0 (1)
Streptococcus spp.	11/9 (20)	Corynebacterium spp.	23/18 (41)	Pseudomonas spp.	4/8 (12)		
Coagulase negative Staphylococcus spp.	3/2 (5)	Gram-positive coccobacilli	2/0 (2)	Gram-negative bacilli	4/5 (9)		
Enterococcus faecium	2/1 (3)	Desemzia incerta	1/0 (1)	Escherichia spp.	5/3 (8)		
Streptomyces spp.	1/1 (2)	Microbacterium spp.	0/1 (1)	E. coli	2/4 (6)		
Brachybacterium paraconglomeratum	1/0 (1)			Pantoea spp.	2/5 (7)		
Macrococcus equiperdus	1/0 (1)			Paenibacillus spp.	1/3 (4)		
				Aeromonas hydrophila	0/3 (3)		
				Gram-negative coccobacilli	2/0 (2)		

				Klebsiella spp.	0/2 (2)		
				Rhizobium spp.	1/0 (1)		
				Alcaligenes faecalis	0/1 (1)		
				Chryseobacterium sp.	0/1 (1)		
				Citrobacter sakazakii	0/1 (1)		
				Comamonas sp.	1/0 (1)		
				Serratia spp.	0/1 (1)		
				Wautersiella spp.	0/1 (1)		
Total	92/88(180)		99/91(190)		34/53(87)		2/1(3)

* Note: 1 horse returned no growth from either eye and there was no growth OS from one horse.

Table 2a. Antimicrobial susceptibility patterns of gram-positive cocci isolated from the normal eyes of horses. Total number (N) and percentage (%) of susceptible isolates for each bacterial species. Median and range (mm) of zone of inhibition is given where no guidelines for breakpoints are known.

Antimicrobial	Staphylococci spp. N=116	Micrococcus spp. N=32	Streptococci spp. N=20	Coagulase negative Staphylococci N=5	Enterococcus spp. N=3	Total Gram-positive cocci N=176
BAC	112 (97%) 17 (0-40)	31 (97%) 25.5 (0-46)	20 (100%) 18 (0-40)	5 (100%) 16 (15-24)	2 (67%) 10 (7-26)	170 (97%)
CEP	99 (85%)	31 (97%)	16 (80%)	5 (100%)	3 (100%) 23 (19-23)	154 (88%)
CHL	111 (96%)	28 (87.5%)	17 (85%)	5 (100%)	3 (100%)	164 (93%)

CIP	109 (94%)	31 (97%)	20 (100%) 24 (15-35)	5 (100%)	3 (100%)	168 (95%)
CLO	98 (84%) 24 (0-34)	29 (88%) 26.5 (0-58)	14 (70%) 24.5 (0-34)	5 (100%) 22 (21-28)	1 (33%) 6 (0-18)	147 (83%)
FA	109 (94%) 21.5 (0-42)	31 (97%) 24 (0-44)	17 (85%) 11 (0-22)	5 (100%) 25 (14-34)	3 (100%) 12 (10-14)	166 (94%)
GEN	99 (85%)	28 (87.5%)	19 (95%) 20.5 (0-36)	5 (100%)	3 (100%) 28 (24-30)	154 (87.5%)
LEV	112 (97%)	29 (91%)	18 (90%)	5 (100%)	3 (100%)	167 (95%)
NEO	116 (100%)	31 (97%)	11 (55%)	5 (100%)	3 (100%)	166 (94%)
OFL	109 (94%)	26 (81%)	18 (90%)	5 (100%)	3 (100%) 16 (15-21)	161 (91%)
TET	99 (85%)	30 (94%)	14 (70%)	5 (100%)	3 (100%)	151 (86%)
TOB	104 (90%)	28 (87.5%)	18 (90%) 19 (0-44)	4 (80%)	3 (100%) 20 (15-24)	157 (89%)

BAC, bacitracin; CEP, cephalothin; CHL, chloramphenicol; CIP, ciprofloxacin; CLO, cloxacillin; FA, fusidic acid; GEN, gentamicin; LEV, levofloxacin; NEO, neomycin, OFL, ofloxacin; TET, tetracycline; TOB, tobramycin.

Table 2b. Antimicrobial susceptibility patterns of gram-positive bacilli and coccobacilli isolated from the normal eyes of horses. Total number (N) and percentage (%) of susceptible isolates for each bacterial species. Median and range (mm) of zone of inhibition is given where no guidelines for breakpoints are known.

Antimicrobial	<i>Bacillus cereus</i> N=80	<i>Bacillus</i> spp. N=65	Gram-positive coccobacilli N=2	Total gram-positive bacilli N=147
BAC	57 (71%) 10 (0-40)	44 (68%) 10 (0-30)	1 (50%) 13 (0-26)	102 (69%)
CEP	11 (14%)	45 (69%)	2 (100%)	58 (39%)
CHL	79 (99%)	61 (94%)	2 (100%)	142 (97%)
CIP	79 (99%)	65 (100%)	2 (100%)	146 (99%)
CLO	22 (28%) 0 (0-32)	53 (82%) 21 (0-34)	1 (50%) 15 (0-30)	76 (52%)
FA	76 (95%) 14 (0-31)	61 (94%) 17 (0-38)	1 (50%) 10.5 (0-21)	138 (94%)
GEN	79 (99%)	64 (98%)	2 (100%)	145 (99%)
LEV	80 (100%)	65 (100%)	2 (100%)	147 (100%)

NEO	79 (99%)	65 (100%)	2 (100%)	146 (99%)
OFL	80 (100%)	65 (100%)	2 (100%)	147 (100%)
TET	64 (80%)	55 (85%)	2 (100%)	121 (82%)
TOB	80 (100%)	65 (100%)	2 (100%)	147 (100%)

BAC, bacitracin; CEP, cephalothin; CHL, chloramphenicol; CIP, ciprofloxacin; CLO, cloxacillin; FA, fusidic acid; GEN, gentamicin; LEV, levofloxacin; NEO, neomycin, OFL, ofloxacin; TET, tetracycline; TOB, tobramycin.

Table 2c. Antimicrobial susceptibility patterns of gram-negative bacilli isolated from the normal eyes of horses. Total number (N) and percentage (%) of susceptible isolates for each bacterial species. Median and range (mm) of zone of inhibition is given where no guidelines for breakpoints are known.

Antimicrobial	Enterobacter spp. N=15	Acinetobacter spp. N=12	Pseudomonas spp. N=11	Escherichia spp. N=8	Gram- negative bacilli N=8	E. coli N=6	Aeromonas hydrophila N=3	Klebsiella spp. N=2	Total gram- negative bacilli N=65
BAC	1 (7%) 0 (0-30)	8 (67%) 14 (0-20)	1 (9%) 0 (0-11)	1 (13%) 0 (0-20)	1 (13%) 0 (0-19)	0 (0%)	0 (0%)	0 (0%)	12 (18%)
CEP	10 (67%)	0 (0%)	1 (9%)	7 (88%)	8 (100%)	6 (100%)	3 (100%) 14 (12-20)	2 (100%)	37 (57%)

CHL	14 (93%)	9 (75%)	7 (64%)	8 (100%)	8 (100%)	6 (100%)	3 (100%)	2 (100%)	57 (88%)
CIP	15 (100%)	12 (100%)	11 (100%)	8 (100%)	8 (100%)	6 (100%)	3 (100%)	2 (100%)	65 (100%)
CLO	1 (7%) 0 (0-40)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)
FA	1 (7%) 0 (0-20)	4 (33%) 0 (0-10)	0 (0%)	8 (100%) 18 (17-26)	2 (25%) 0 (0-18)	0 (0%)	0 (0%)	0 (0%)	15 (23%)
GEN	14 (93%)	12 (100%)	11 (100%)	8 (100%)	8 (100%)	5 (83%)	3 (100%)	2 (100%)	63 (97%)
LEV	14 (93%)	12 (100%)	11 (100%)	8 (100%)	8 (100%)	6 (100%)	3 (100%)	2 (100%)	64 (98%)
NEO	12 (80%)	11 (92%)	11 (100%)	8 (100%)	8 (100%)	5 (83%)	3 (100%)	2 (100%)	60 (92%)
OFL	15 (100%) 30 (25-36)	11 (92%) 26.5 (0-38)	11 (100%)	8 (100%) 28.5 (22-32)	8 (100%) 30 (25-35)	6 (100%) 32 (29-33)	3 (100%) 33 (28-36)	2 (100%) 25.5 (25-26)	64 (98%)
TET	14 (93%)	12 (100%)	10 (91%)	8 (100%)	7 (87.5%)	5 (83%)	3 (100%)	2 (100%)	61 (94%)
TOB	15 (100%)	12 (100%)	11 (100%)	8 (100%)	8 (100%)	6 (100%) 18 (12-20)	3 (100%)	2 (100%)	65 (100%)

BAC, bacitracin; CEP, cephalothin; CHL, chloramphenicol; CIP, ciprofloxacin; CLO, cloxacillin; FA, fusidic acid; GEN, gentamicin; LEV, levofloxacin; NEO, neomycin, OFL, ofloxacin; TET, tetracycline; TOB, tobramycin.

Note: that although 9 isolates of gram-negative bacilli were identified, only 8 could be recovered for susceptibility testing.

Table 3. Fungal types and species reported as number of isolates OD, OS and OU for 111 eyes from 73 horses*. A total of 78 fungal isolates were identified as either molds (67) or yeasts (11) from 60 horses.

Mold species	Number OD/OS (OU)	Yeast species	Number OD/OS (OU)
Penicillium spp.	6/7 (13)	Rhodotorula spp.	1/4 (5)
Aspergillus spp.	8/4 (12)	Candida spp.	1/2 (3)
Scopulariopsis spp.	4/4 (8)	Toropulopsis spp.	1/1 (2)
Trichophyton spp.	4/1 (5)	Cryptococcus sp.	0/1 (1)
Chrysosporium spp.	1/3 (4)		
Drechslera spp.	1/3 (4)		
Fusarium spp.	3/1 (4)		
Microsporum spp.	2/2 (4)		
Geotrichium spp.	1/2 (3)		
Madurella spp.	1/2 (3)		
Cladosporium spp.	1/1 (2)		
Mucor spp.	1/1 (2)		
Curvularia sp.	0/1 (1)		
Exophiala sp.	0/1 (1)		
Sporidiobolus sp.	0/1 (1)		
Total	33/34 (67)		3/8 (11)

Note: the fungal growth from 13 horses were not identified, fungi were isolated unilaterally from 35 horses, bilaterally from 38 horses and none were isolated from either eye in 22 horses.

Table 4a. Antifungal susceptibility patterns of molds isolated from the normal eyes of horses. Total number (N) and percentage (%) of susceptible isolates for each species. Median and range (mm) of zone of inhibition is given where no guidelines for breakpoints are known.

Antifungal	Penicillium spp. N=13	Aspergillus spp. N=12	Scopulariopsis spp. N=8	Trichophyton spp. N=5	Chrysosporium spp. N=4	Drechslera spp. N=4	Fusarium spp. N=4	Microsporum spp. N=4
FLU	7 (54%)	3 (25%)	4 (50%)	4 (80%)	4 (100%)	4 (100%)	4 (100%)	4 (100%)

	10 (0-23)	0 (19-25)	5 (0-14)	18 (0-44)	18 (8-50)	30 (27-38)	23.5 (20-41)	19 (16-25)
ITR	13 (100%) 26 (11-34)	12 (100%) 23.5 (12-32)	4 (50%) 5.5 (0-25)	5 (100%) 28 (20-34)	3 (75%) 19 (7-60)	4 (100%) 42 (35-45)	4 (100%) 27.5 (26-41)	4 (100%) 30.5 (22-48)
KET	13 (100%) 23 (11-55)	11 (92%) 20.5 (0-42)	8 (100%) 20 (12-34)	5 (100%) 38 (23-40)	2 (50%) 12.5 (0-35)	4 (100%) 52 (50-55)	4 (100%) 38.5 (30-43)	4 (100%) 37 (30-86)
MIC	13 (100%) 15.5 (11-26)	12 (100%) 13.5 (12-29)	7 (88%) 12.5 (0-22)	5 (100%) 26 (13-40)	2 (50%) 8 (0-30)	4 (100%) 34.5 (34-35)	4 (100%) 38 (33-40)	4 (100%) 52 (25-60)
VOR	12 (92%) 30 (0-48)	12 (100%) 36 (11-45)	6 (75%) 11.5 (0-37)	5 (100%) 32 (30-40)	3 (75%) 17 (0-30)	4 (100%) 53.5 (40-55)	4 (100%) 33 926-46)	4 (100%) 41 (20-60)

Antifungal	Geotrichum spp. N=3	Madurella spp. N=3	Cladosporium spp. N=2	Mucor spp. N=2	Curvularia sp. N=1	Exophiala sp. N=1	Sporidiobolus sp. N=1	Total N=67
FLU	3 (100%) 30 (26-30)	3 (100%) 40 (32-70)	1 (50%) 10.5 (0-21)	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	42 (62.7%) 14.5 (0-70)
ITR	3 (100%) 23 (20-23)	3 (100%) 48 (40-64)	1 (50%) 11.5 (0-23)	1 (50%) 8 (0-16)	1 (100%) 27	1 (100%) 24	1 (100%) 20	61 (91%) 24 (0-64)
KET	3 (100%) 32 (28-34)	3 (100%) 58 (52-64)	2 (100%) 18 (16-20)	1 (50%) 7 (0-14)	1 (100%) 14	1 (100%) 20	1 (100%) 40	64 (95.5%) 24.5 (0-86)
MIC	3 (100%) 27 (25-28)	3 (100%) 50 (34-64)	1 (50%) 7 (0-14)	1 (50%) 5 (0-10)	0 (0%) 0	1 (100%) 14	1 (100%) 18	62 (92.5%) 18 (0-64)
VOR	3 (100%) 32 (32-33)	3 (100%) 52 (45-60)	2 (100%) 14 (11-17)	0 (0%) 0	0 (0%) 0	1 (100%) 32	1 (100%) 40	62 (92.5%) 32 (0-60)

FLU, fluconazole; ITR, itraconazole; KET, ketoconazole; MIC, miconazole; VOR, voriconazole

Table 4b. Antifungal susceptibility patterns of yeasts isolated from the normal eyes of horses. Total number (N) and percentage (%) of susceptible isolates for each species. Median and range (mm) of zone of inhibition is given where no guidelines for breakpoints are known.

Antifungal	Rhodotorula spp. N=5	Candida spp. N=3	Torulopsis spp. N=2	Cryptococcus sp. N=1	Total N=11
FLU	1 (20%) 0 (0-16)	2 (67%) 25.5 (0-40)	1 (50%) 11 (0-22)	1 (100%) 14	5 (45%) 0 (0-40)
ITR	2 (40%) 0 (0-50)	2 (67%) 21 (0-30)	2 (100%) 21.5 (9-34)	1 (100%) 21	8 (73%) 14 (0-50)
KET	5 (100%) 45 (3-50)	3 (100%) 36 (15-102)	2 (100%) 31.5 (13-45)	1 (100%) 21	11 (100%) 45 (15-102)
MIC	3 (60%) 11 (0-18)	2 (67%) 32 (0-88)	2 (100%) 11.75 (11.5-12)	1 (100%) 18	8 (73%) 12 (0-88)
VOR	4 (80%) 16 (0-36)	3 (100%) 32 (25-40)	1 (50%) 7.5 (0-15)	1 (100%) 25	9 (82%) 23 (0-40)

FLU, fluconazole; ITR, itraconazole; KET, ketoconazole; MIC, miconazole; VOR, voriconazole

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