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ORIGINAL REPORT

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Efficacy of 0.2% povidone-iodine and 0.1% polyhexamethylene biguanide as preoperative antiseptics in equine ophthalmic procedures

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Abstract

Objective: This retrospective study evaluates the efficacy of povidone-iodine (PI) and polyhexamethylene biguanide (PHMB) as preoperative antiseptics in equine ophthalmic procedures.

Animals Studied: Horses that underwent routine ophthalmic surgery and procedures.

Procedures: Data were collected retrospectively from the medical records of equine patients undergoing ophthalmic procedures. Inclusion criteria were sampling for aerobic microbial culture at three different time points (T0: pre-irrigation, T1: post-irrigation, and T2: postoperatively) and T0 showing bacterial growth. Microbiological outcomes were assessed semi-quantitatively by creating a scoring system to describe the bacterial load. Furthermore, the species detected were evaluated. Poisson regression analysis was performed to evaluate the efficacy of the disinfectants.

Results: Eighty eyes (75 horses) met the inclusion criteria, with 36 cases being aseptically prepared with PI and 44 with PHMB. Both antiseptics significantly reduced the bacterial load and number of bacterial species ($p < .001$) between time points T0 and T1, and T0 and T2. PHMB showed a reduction in the bacterial load by 64% (CI: 51%–73%) whereas PI reduced it by 48% (CI: 36%–58%) between time points T0 and T1. The reduction in the number of bacterial species between time points T0 to T1 was significantly greater in the PHMB group (85%, CI: 70%–93%), compared to PI (47%, CI: 26%–62%).

Conclusion: Both PHMB and PI reduced the bacterial load and number of species on the ocular surface and eyelids significantly, with 0.1% PHMB being superior to 0.2% PI. Therefore, PHMB can be considered as a good alternative in preoperative antisepsis in equine ophthalmic procedures.

KEYWORDS

antisepsis, horse, ocular surface, PI, polyhexanide, preoperative disinfection

1 | INTRODUCTION

Commensal organisms of the ocular microbiome may become pathogenic and cause infection after surgical intervention.[1] Therefore, aseptic preparation protocols are recommended in minimizing the risk of postoperative endophthalmitis[2,3] and surgical site infections.

Various methods and compounds have been used to minimize postoperative complications related to infection. An ideal disinfectant should have a broad antimicrobial activity, a quick onset of action, and should be nonirritating and nontoxic to the patient. Due to the vulnerable ocular structures, the safety and tolerability of antiseptics play a major role when selecting the appropriate disinfectant.[4–6]

Povidone-iodine (PI) has a wide antibacterial spectrum and activity not only against antibiotic-resistant, but also antiseptic-resistant strains.[7,8] It has been shown in vitro and ex vivo that PI has an anti-biofilm activity against mature bacterial and fungal biofilms.[9–12] Furthermore, the virucidal effect of PI has also been demonstrated in a wide range of enveloped and non-enveloped viruses.[13,14] Antifungal,[15,16] antiprotozoal,[17] and, with longer exposure times, sporicidal properties of PI[18] have also been described.

The exact mechanism of action of PI is still unknown. It is presumed that free iodine molecules are released from the povidone-iodine complex and destroy key proteins, nucleotides, and fatty acids after briskly penetrating microorganisms.[19]

Diluted PI has a quicker onset of bactericidal action than the 10% PI stock solution, as dilution increases the free iodine available.[20,21] Several in vitro studies have demonstrated the antimicrobial and antiviral activities of PI concentrations under 10%.[5,22–24] However, an in vivo toxicity study in rabbits showed that even a 1% PI solution can cause damage to the corneal epithelium.[25] The corneal endothelium is even more sensitive, with a PI concentration of 0.1% solution being capable of causing significant damage.[26]

Roberts et al. conducted an in vivo study of the effectiveness of diluted PI on the canine ocular surface. 0.2% PI was shown to be equally bactericidal as the 1% and 5% dilutions. As a consequence, the use of 0.2% aqueous solution of PI was recommended as a preoperative ocular surface antiseptic[27] and most textbooks dealing with veterinary ophthalmology recommend this concentration.[28,29]

Polyhexamethylene biguanide (PHMB; polyhexanide), like PI, is a potent biocide with a broad antimicrobial spectrum. Its antibacterial,[30] antifungal[31] properties and also its activity against fungal and bacterial biofilms have been described.[32] Its virucidal activity on enveloped and non-enveloped viruses has also been shown.[33,34] In addition, PHMB is a widely used and effective biocide in the treatment of Acanthamoeba keratitis in humans.[35] The specific mechanism of action of PHMB has been recently described: PHMB selectively binds and condenses bacterial chromosomes and exhibits distinctive access to bacterial and mammalian cellular DNA. Acquired resistance to PHMB has not been reported and selective DNA condensation furnishes an unforeseen model for antimicrobial action that may avoid the development of resistance.[30]

Studies examining the safety and tolerability of topical conjunctival instillation of 0.08% PHMB in rabbits and humans did not show any side effects.[36] Nevertheless, a recent report in the human literature documented a case series, where an accidentally high concentration of PHMB (0.2%) caused irreversible damage to the eyes.[37]

To the authors' knowledge, the efficacy of PI and PHMB as preoperative antiseptics has not yet been described in horses. Therefore, the aim of this retrospective cohort study is to compare the efficacy of the two routinely used aqueous disinfectant solutions in reducing the bacterial load and bacterial species of the ocular surface and eyelids in horses undergoing ophthalmic procedures at the Equine Clinic, Freie Universität Berlin, Germany.

2 | MATERIALS AND METHODS

2.1 | Case selection

Medical records of horses undergoing ophthalmic surgery and procedures from October 2016 until April 2021 were evaluated retrospectively. All animals were privately owned and had routine ophthalmic procedures either in standing sedation or general anesthesia at the Equine Clinic, Freie Universität Berlin. At the time of patient admission, all clients gave consent for the use of medical data contained in the medical records for research and teaching purposes. Indication for surgery or procedure was based on the clinical diagnosis of the horses by a board-certified veterinary ophthalmologist or resident and not related to this analysis. Inclusion criteria were having three perioperative microbiological swabs cultured, with the samples prior antiseptic preparation showing bacterial growth. Recorded parameters included age at surgery or procedure, sex, breed, ocular disease, ocular surgery or procedure performed, disinfectant used for preoperative antisepsis and preoperative topical antibiotic treatment.

2.2 | Sample collection and processing

Preoperative disinfection was performed with commercially available povidone-iodine 10% solution (Braunol®,

B. Braun) diluted with sterile 0.9% saline (NaCl 0.9%, B. Braun) in a 1:50 ratio to achieve the desired 0.2% concentration until August 2019 (Group 1).

Due to a change in the facility's hygiene protocol and based on promising reports from human ophthalmology,[38] 0.1% polyaminopropyl biguanide (PHMB, Vet Care ProntoVet irrigation solution, B. Braun Vet Care GmbH) was used for aseptic preparation from September 2019 onward (Group 2).

During the whole period, as part of a routine perioperative hygiene protocol three samples were taken from each eye. The first sample was collected pre-irrigation (T0), and the second sample post-irrigation and aseptic preparation (T1) and the third sample postoperatively (T2) before the surgical draping were removed. Samples were taken using a swab (TS-SWAB, Sterile Transport Swab, Heinz Herenz) of which the cotton tip applicator was premoistened with sterile saline solution. The swab was rolled multiple times back and forth in the inferior and superior conjunctival fornix as well as on the upper and lower eyelid margin.

The preoperative aseptic protocol was conducted in the following manner: The ocular structures were first irrigated with 100 mL of sterile saline solution using 20 mL syringes and 20-gauge needles, with the shaft being removed from the hub in order to increase the pressure during the lavage. Von Graefe forceps were used to elevate and retract the third eyelid in order to successfully cleanse all parts of the ocular surface and conjunctival fornices. Next, the eyelids and surrounding skin were prepared aseptically with surgical swabs soaked in either 0.2% PI or 0.1% PHMB.

The swabs were kept refrigerated (<8°C) and were brought within 24 h to the Institute of Microbiology and Epizootics, Freie Universität Berlin, Germany, for microbial analysis.

2.3 | Bacteriological examination

The collected swabs were inoculated for the detection of aerobic bacteria on Columbia blood agar (5% sheep blood), Gassner agar, and Brilliance UTI Clarity agar plates (Thermo Scientific). Twenty-four to 48 h after cultivation under aerobic conditions at 36°C, bacterial species were identified by evaluating colony morphology and via matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS)-based identification with Bruker ultrafleXtreme in combination with flexControl (Version 3.4) and MBT Compass (Version 4.1) software (Bruker Daltonics). The semi-quantitative assessment of bacterial growth took place using the following classification system: +++ "heavy" (>100 colony-forming units (CFU) grown per agar plate), ++ "moderate" (up to 100 CFU/plate), + "low" (up to 30 CFU/plate), and ± "scanty" (up to 5 CFU/plate). To allow quantitative comparison of the results, we created a scoring system of the amount of growth seen as follows: no growth = 1, scanty growth = 2, low growth = 3, moderate growth = 4, and heavy growth = 5. If more than one bacterial species were detected in a sample, the scoring results of the individual species were summed (e.g., a sample with two species with moderate growth, received a score of 8).

2.4 | Statistical analysis

Analyses were performed with SPSS (IBM® SPSS® Statistics, Version 28). In order to be able to measure the reduction in microbial growth, cases with a negative culture at T0 were excluded from the statistical analysis.

The semi-quantification of bacteria and the number of species were analyzed descriptively grouped by disinfectant and time point. The data were not normally distributed due to zero inflation.

To assess differences of the bacterial load and number of species cultured from the ocular surface between the disinfectants at the three different time points, Poisson regression analysis was performed.

Two models were calculated for the outcomes "bacterial load" and "number of different species." The disinfectant and the interaction of disinfectant and time point were included as fixed effects. Therefore, the time point was set as an offset variable to account for the different amount of bacterial load and number of species before the disinfection. Thereby, the relative reduction caused by each disinfectant over time was estimated and compared to each other. Significance was set at a value of $p < .05$.

3 | RESULTS

3.1 | Equine patients

Between October 2016 and April 2021, a total of 188 ophthalmic procedures were performed on 176 horses, with some horses undergoing bilateral ocular procedures. Out of these cases, 99 eyes of 89 equine patients had a complete set of three swabs cultured. Nineteen cases were excluded because of a negative bacterial growth at T0, resulting in a total of 80 eyes of 75 horses.

In 36 cases, the preoperative antisepsis was performed using 0.2% PI (Group 1) and in 44 cases with 0.1% PHMB (Group 2).

The equine patients had a mean age of 13.6 ± 5.7 years of age (range: 3.9 to 25.5 years). There were 36 (45.0%) mares, 37 (46.3%) geldings, and 7 (8.7%) stallions.

The study population included 31 different horse breeds with the Icelandic horse (8/80, 10.0%), Appaloosa (7/80, 8.8%), Haflinger (5/80, 6.3%), Hanoverian (5/80, 6.3%), and Mecklenburger (5/80, 6.3%) being most commonly presented. In 7 cases (8.8%), the breed was not specified.

The indications for an ophthalmic procedure or surgery included the following diseases: equine recurrent uveitis (36/80, 45.0%), ulcerative keratitis (12/80, 15.0%), non-ulcerative keratitis (9/80, 11.3%), conjunctival squamous cell carcinoma with or without involvement with the nictitating membrane (8/80, 10.0%), corneal squamous cell carcinoma with or without the involvement of the conjunctiva (7/80, 8.8%), conjunctival mass (2/80, 2.5%), endothelial dysfunction (2/80, 2.5%), and one (1.3%) case each of intraocular mass, heterochromic iridocyclitis, periocular mass, and lid laceration.

The following procedures were performed: anterior chamber paracentesis (ACP) (17/80, 21.3%), excision or biopsy of periocular mass (14/80, 17.5%), corneal grafting procedure (10/80, 12.5%), vitrectomy (9/80, 11.3%), suprachoroidal cyclosporine A implant (8/80, 10.0%), enucleation (6/80, 7.5%), lamellar keratoplasty (3/80, 3.8%), diamond burr debridement of the cornea (3/80, 3.8%), penetrating keratoplasty (2/80, 2.5%), intravitreal injection (2/80, 2.5%), placement of subpalpebral lavage system (2/80, 2.5%), eyelid surgery (2/80, 2.5%), subconjunctival cyclosporine A implant (1/80, 1.3%), and corneal laceration repair (1/80, 1.3%).

In the PI group, the procedures were performed under general anesthesia in 11 (30.6%) cases and with standing sedation in 25 (69.4%) cases. In the PHMB group, 31 (70.5%) procedures were performed under general anesthesia and 13 (29.5%) in standing sedation.

In Group 1, 17 (47.2%) cases were pretreated with topical antibiotics, 16 (44.4%) cases were not pretreated with topical antibiotics and in 3 (8.3%) cases it was unknown if pretreatment took place. In Group 2, 31 (70.5%) cases were pretreated with topical antibiotics and 13 (29.5%) were not pretreated with topical antibiotics. All in all, 46/80 (57.5%) eyes were treated with topical antibiotics prior to procedure and sample collection.

Altogether, 266 different bacterial isolates with a wide range of bacterial species from 17 families were cultured in this study. The different families with the number of species isolated are displayed in Table 1.

TABLE 1 Absolute number of bacterial isolates categorized in families obtained from the ocular surface and eyelids of horses presented for ocular surgery and procedures ($n = 80$).

Family	Time point T0		Time point T1		Time point T2	
	Number of isolates	Percent (%)	Number of isolates	Percent (%)	Number of isolates	Percent (%)
Staphylococcaceae	60	38	20	32.8	19	40.4
Bacillaceae	45	28.5	19	31.1	12	25.5
Streptococcaceae	13	8.2	10	16.4	7	14.9
Moraxellaceae	6	3.8	–	–	2	4.3
Pasteurellaceae	6	3.8	4	6.6	1	2.1
Corynebacteriaceae	5	3.2	–	–	–	–
Erwiniaceae	5	3.2	1	1.6	1	2.1
Micrococcaceae	5	3.2	1	1.6	2	4.3
Enterobacteriaceae	3	1.9	2	3.3	–	–
Streptomycetaceae	3	1.9	–	–	–	–
Aerococcaceae	2	1.3	–	–	–	–
Enterococcaceae	2	1.3	1	1.6	–	–
Lactobacillaceae	2	1.3	-	-	–	–
Microbactariaceae	1	0.6	1	1.6	–	–
Flavobacteriaceae	–	–	1	1.6	1	2.1
Morganellaceae	–	–	1	1.6	1	2.1
Alcaligenaceae	–	–	–	–	1	21
Total No. of isolates	158	100	61	100	47	100

Abbreviations: T0, pre-irrigation; T1, post-irrigation; T2, postoperatively.

3.2 | Efficacy of PI and PHMB

Results of the efficacy of the disinfectants in the two different time points (T1, T2) are shown in Figure 1 and Table 2A,B. Both disinfectants reduced the bacterial load and number of species between the time points T0 and T1 significantly ($p < .001$). This significant reduction was true in both groups also when comparing T0 to T2. However, in two cases in each group, a mild recontamination with scanty bacterial growth (score 2) was observed postoperatively.

After disinfection, there was a complete elimination of bacterial load at T1 in 6 (16.7%) and in 36 (81.8%) cases in Group 1 and Group 2, respectively. At the T2 time point, there was no bacterial growth detected on the culture plates in 11 (30.6%) and in 35 (79.5%) cases in Group 1 and Group 2, respectively.

Even though the ocular surface and eyelids at time point T0 (pre-irrigation) in Group 2 were less contaminated compared to Group 1, PHMB exceeded PI in reducing the bacterial load. PHMB reduced the bacterial load by 64% (CI: 51%–73%) whereas PI by 48% (CI: 36%–58%) from time point T0 to T1 (Table 2). As the confidence intervals overlap, this difference was not significant.

Concerning the reduction in the number of species, PHMB performed significantly better than PI: PHMB reduced the number of species by 85% (CI: 70%–93%) and PI by 47% (CI: 26%–62%; Table 2B).

4 | DISCUSSION

In this retrospective analysis, we investigated and compared the efficacy of two different routinely used antiseptic preparations on the ocular surface and eyelids of horses for the first time. The microbiology results clearly show a good antimicrobial efficacy of both 0.2% povidone-iodine and 0.1% polyhexamethylene biguanide solution

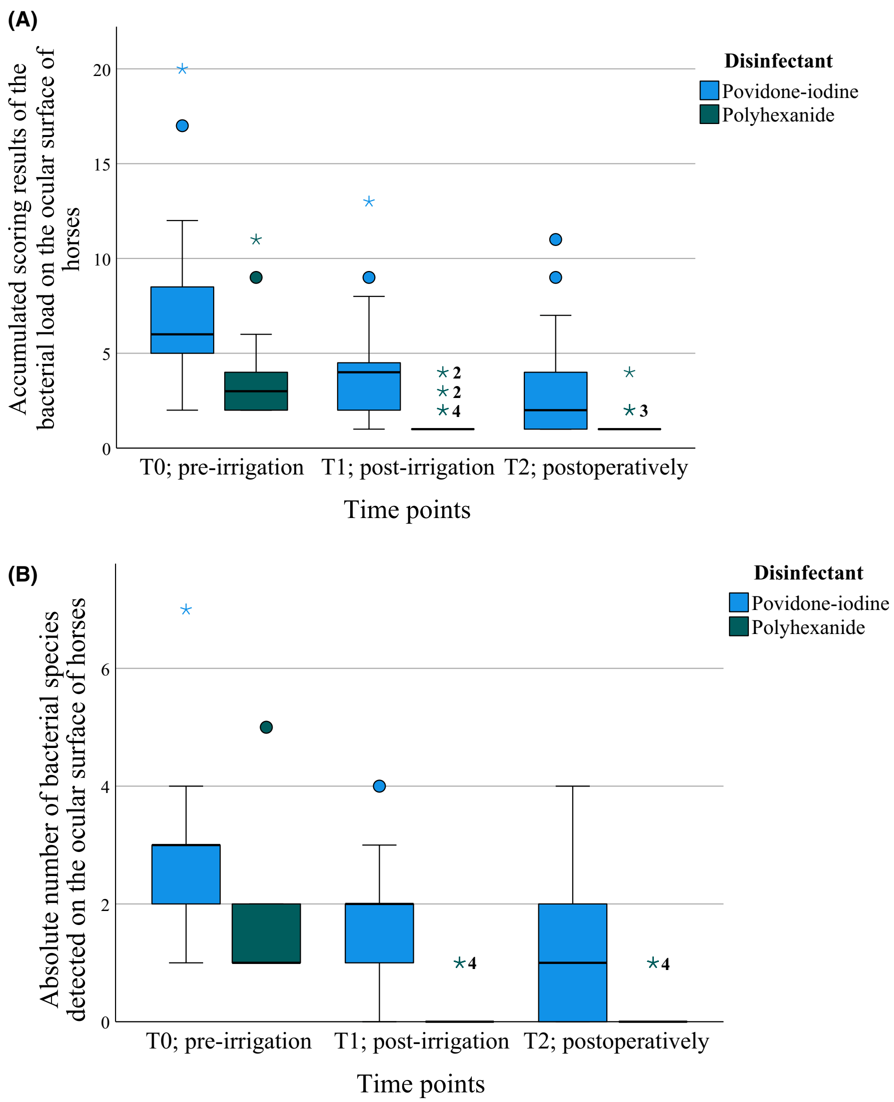

FIGURE 1 Grouped boxplots representing (A) the changes in the accumulated scoring results for the bacterial load including the amount of growth and (B) the changes in the number of bacterial species detected on the ocular surface ignoring the intensity of growth at different time points depending on the disinfectant used. The dots (°) represent mild outliners, that have scores greater than the upper quartile range plus 1.5 times the interquartile range. The asterisk (*) depict extreme outliners, with scores greater than the upper quartile plus three times the interquartile range. By some extreme outliers, there were multiple cases with the same value which were overlapping. The numbers next to these outliers represent the number of cases affected.

TABLE 2 The Poisson linear regression model represents the amount of reduction achieved by PHMB and PI in the (A) bacterial load and (B) number of species detected at the different time points.

(A)				
Parameter Estimates				
		95% Wald Confidence Interval for Exp (B)		
Parameter	**Exp (B)**	**Lower**	**Upper**	**Significance**
(Intercept)	7.143	6.310	8.085	<.001
PHMB	0.509	0.417	0.621	<.001
PI	1	–	–	
Bacterial load in the PHMB group				
T0	1	–	–	
T0–T1	0.362	0.268	0.490	<.001
T0–T2	0.344	0.253	0.467	<.001
Bacterial load in the PI group				
T0	1	–	–	
T0-T1	0.516	0.417	0.638	<.001
T0-T2	0.384	0.303	0.486	<.001
(B)				
Parameter Estimates				
		95% Wald Confidence Interval for Exp (B)		
Parameter	**Exp (B)**	**Lower**	**Upper**	**Significance**
(Intercept)	2.800	2.297	3.413	
PHMB	0.495	0.360	0.682	<.001
PI	1	–	–	<.001
Number of species in the PHMB group				
T0	1	–	–	
T0–T1	0.148	0.073	0.297	<.001
T0–T2	0.164	0.084	0.320	<.001
Number of species in the PI group				
T0	1	–	–	
T0–T1	0.531	0.379	0.743	<.001
T0–T2	0.388	0.267	0.564	<.001

Abbreviations: PHMB, polyhexamethylene biguanide; PI, povidone-iodine; T0, pre-irrigation; T1, post-irrigation; T2, postoperatively.

(polyhexanide) on aerobic bacteria. A tendency toward a difference between the two disinfectants was observed, with PHMB being more effective.

Generally, PI is the most widely used antiseptic agent in human[21] and in veterinary ophthalmology,[27] even though some in vivo studies did not show a significant reduction in positive bacterial cultures when utilizing PI as a disinfectant agent.[39,40]

Within the literature, there are different recommendations concerning the concentration of PI and exposure times required. One study showed a significantly better reduction with a concentration of 5% PI compared to 1%, with a 1-min contact time,[41] yet another study advised a 3-min contact time with a 5% PI solution prior to cataract surgery.[3] Along these recommendations, a study can be found, which rather advises the repetitive pre- and intra-operative surface irrigation of 0.25% PI during cataract surgery.[42] Choosing to follow the findings of Roberts et al., the authors decided to use the 0.2% PI concentration.[27] The contact time of the antiseptic was not standardized—the eyelids and periocular skin were wiped until the surgical swabs shone macroscopically clean.

The surgeon needs to be aware that PI may have some drawbacks. Although not published in the veterinary literature, allergic reaction to PI is a side effect reported in the human literature.[17] York et al. have demonstrated that concentrations of 0.5% and higher delay corneal and conjunctival wound healing in rabbit models.[43] Additionally, repeated application of PI may cause symptoms of dry eye disease in humans.[44] Therefore, caution is advised in patients displaying signs of tear film disorders and with patients undergoing multiple ocular surgeries or procedures.

PI should not be used in cases of corneal or globe perforation especially if the aim is the salvage of the globe, as it has been shown, that intraocular concentrations greater than 0.1% are endotheliotoxic.[25,26,45] Furthermore, PI has been reported to be potentially retinotoxic, thereby unintended leakage into the globe should be avoided.[46]

PI induced thyroid dysfunction has been reported in the human literature.[47] Although to the authors' knowledge no such reports exist in the veterinary literature, the authors recommend that caution should be taken when considering this disinfectant agent on open wounds and lacerations in patients with a known hypo- or hyper-thyroidism.

Due to the aforementioned reasons and to avoid triggering adverse reactions of the delicate ocular structures, the authors based their disinfection protocol described by Stades et al. and did not expose the ocular surface to the active agent.[48] During the cleansing of the eyelids, a minimal amount of the antiseptic may have leaked onto the ocular surface, but deliberate irrigation of these structures with the antiseptic agent did not take place.

Antiseptics such as PHMB, chlorhexidine,[49] picloxydine,[50] and ozonized oil[51] are being studied in human ophthalmology as alternatives to PI. Still, there is not a large number of in vivo studies on the use of PHMB as an antiseptic agent neither in human nor in veterinary ophthalmology. The authors started using PHMB as an antiseptic agent in 2019 after changing the hygiene protocol and reading the study of Hansmann et al.[38] In that study, the authors recorded a significant reduction in the colony-forming units on the ocular surface with both PI and PHMB, did not see a significant difference in side effects comparing the two disinfectants, and documented a longer lasting reduction in CFUs on the ocular surface with PHMB.[38] Considering the efficacy of PI and PHMB, our results are similar with Hansmann et al., as both antiseptics significantly reduced the bacterial load and number of aerobic bacterial species on the ocular surface and eyelids of horses.

The results show a noticeably higher bacterial load at the time point T0 in the PI group than in the PHMB group. The reasons of this difference can only be speculated. The underlying ophthalmic disease does not seem to have a major influence, as both groups show equal numbers of ocular diseases presumed to have a higher degree of contamination (e.g., corneal ulcer, eyelid laceration, Group 1 7/36, Group 2 9/44). Between 2016 and 2019, a major renovation of the Equine Clinic took place. This might may have caused a difference in the initial bacterial load, due to the complete decontamination of the buildings. Furthermore, samples were taken by different personnel between 2016 and 2021, which may have resulted in the different outcomes, even though the sampling technique was standardized. Moreover, in Group 2 more patients received topical antibiotics before procedure, which may have caused the lower bacterial load at time point T0. Whether a bactericidal or bacteriostatic antibiotic was used may also have influenced the results. Lastly, the procedure or surgery was either done in standing sedation or general anesthesia. Huppes et al. have previously shown that surgical site infection is higher in procedures performed in standing sedation.[52] This might have contributed to the higher bacterial load in Group 1, as more than two-thirds of these procedures were performed in standing sedation.

Although in the human literature a study reported no complications after ACP without any preoperative antisepsis,[53] there is a case report on bacterial endophthalmitis after ACP,[54] and the incidence of post-intravitreal injection has been reported to range from 0.028% to 0.056% per injection.[55,56] Therefore, the authors decided to carry out the aseptic preparation before conducting an ACP.

There are several limitations due to the retrospective nature of this data analysis. Horses were not randomly assigned to the two different groups, but by a change in the hygiene protocol of the clinic. In order to reach sufficient numbers, all patients undergoing any kind of ophthalmic procedure were included. As a consequence, there was a great diversity of diseases and procedures; however, these were not evenly distributed within the groups, with some cases being very few in number. Therefore, reliable statistics concerning specific diseases, surgical procedures, and/or duration of surgeries or procedures were not possible. Last but not least, there is no control group. With respect to the patient's welfare and good clinical practice, all patients underwent preoperative aseptic preparation. A randomized comparison with an untreated control group would only be possible in a prospective, registered animal experiment.

More than half of the patients (57.5%) were treated with topical antibiotics prior to sample collection. In theory, this may have disrupted the normal equine conjunctival bacterial microbiome and influenced our culture results. However, the cultured conjunctival flora seen in this study was similar to those in previously reported studies.[48,49] Besides, the authors assume that an antibiotic treatment had no effect on the efficacy of the antiseptic used, as antibiotics are not described to interfere with biocides.

We cannot neglect the fact that our routine hygiene protocol used traditional culture-based methods to semi-quantitatively assess the bacterial load. We aimed to take representative swabs from the ocular surface, but we did not use a standardized volume (e.g., to flush the ocular surface) and/or standardized contact time to allow quantitative analysis of the bacterial growth (CFU/mL). Within this report, only the results of aerobic microbial cultures are shown. Due to changes within the hygiene protocol, not all samples underwent additional anaerobic culture and analysis for fungal growth. The reason for the change in the hospital protocol was the following: low detection rates in both anaerobic and fungal species; no evidence for surgical site infections and the aim to reduce laboratory costs for animal owners (data not shown).

Lafrenz et al. have shown in 2020 in a pilot study that culture-independent methods are more effective to describe the conjunctival microbiota community of the equine eye.[57] However, the use of these methods in the everyday clinical setting have still some challenges and classical bacterial isolates are still required for antimicrobial resistance testing.

The use of a more sophisticated method would have been considered, if the present study was a prospective study. As in the current analysis bacterial growth on the culture plates was semi-quantitatively measured, we had to create a novel scoring system to be able to analyze the results. With quantitative measurements, the obtained data could have been more precisely analyzed.

To the authors' knowledge, this is the first report in veterinary literature, which compared the antiseptic effect of 0.2% PI and 0.1% PHMB on the ocular surface of horses. The present study provides evidence that both antiseptics are effective in reducing the bacterial load on the ocular surface and eyelids, but with a greater decline being achieved with PHMB. The commercially available and inexpensive PHMB should be considered as a good alternative for PI in the field of ophthalmic surgery.

AUTHOR CONTRIBUTIONS

Anna Farkas: Data curation; formal analysis; investigation; visualization; writing – original draft. **Katharina Thieme:** Data curation; investigation; writing – review and editing. **Tanawan Soimala:** Investigation; methodology. **Charlotte K. Jensen:** Formal analysis; validation; writing – review and editing. **J. Corinna Eule:** Conceptualization; methodology; project administration; supervision; writing – review and editing.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

None.

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