Small Animal Clinic, Department of Veterinary Medicine

Freie Universität Berlin

Hygiene in Ophthalmic Surgery and Bacterial Resistance on the Ocular Surface of Animals

Thesis submitted for the fulfillment of
a Doctor medicinae veterinariariae (Dr. med. vet.) degree
in the Faculty of Veterinary Medicine
at the
Freie Universität Berlin

Submitted by
Tanawan Soimala
Veterinarian from Bangkok, Thailand

Berlin 2018
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Dean:	Univ.-Prof. Dr. Jürgen Zentek
First Reviewer:	Univ.-Prof. Dr. Johanna Corinna Eule
Second Reviewer:	Univ.-Prof. Dr. Marcus Fulde
Third Reviewer:	Univ.-Prof. Dr. Leo Brunnberg

Descriptors *(according to CAB-Thesaurus):*

horses, cats, dogs, conjunctiva, methicillin resistant staphylococcus aureus, microbiota (MeSH), povidone-iodine (MeSH), PFGE, eye diseases

Day of Doctorate: 13.07.2018

Bibliografische Information der *Deutschen Nationalbibliothek*
Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über <https://dnb.de> abrufbar.

ISBN: 978-3-86387-919-8
Zugl.: Berlin, Freie Univ., Diss., 2018
Dissertation, Freie Universität Berlin
D188

 Choriner Str. 85 - 10119 Berlin
verlag@menschundbuch.de – www.menschundbuch.de

For important peoples in my life

ขอบคุณ Prof. Dr. Corinna Eule ที่เป็นแบบอย่างที่ดี แก่ศิษย์คนนี้ ผลักดัน ใส่ใจอย่างสุดความสามารถ ขอบคุณสำหรับทุกสิ่งทุกอย่าง ที่ทำให้วิทยานิพนธ์ฉบับนี้สำเร็จออกมาในที่สุด

ขอบคุณ Prof. Dr. Leo Brunnberg คุณครูผู้ให้โอกาสเด็กไทยคนนี้ ตั้งแต่ก้าวแรก ทั้งความช่วยเหลือด้านวิชาการ ด้านความร่วมมือระหว่างคณะ จนถึงวันสอบจบ

ขอบคุณ รองศาสตราจารย์ อุษา เชษฐานนท์ ที่ช่วยผลักดันตั้งแต่วินาทีแรก อาจารย์เป็นดังต้นไม้ใหญ่ แผ่กิ่งก้านใบที่อบอุ่น ให้ร่มเงาแก่ลูกคนนี้ คอยให้กำลังใจ ทำให้ข้าพเจ้าผ่านจุดที่ยากที่สุดมาได้ในที่สุด

ขอบคุณ คุณแม่ สุวภา สร้อยมาลา และ คุณพ่อ ทวีวัช สร้อยมาลา ที่ให้ชีวิต และเลี้ยงดูด้วยความรัก นายสาคร ชุ่มชื่น และนาง ระเบียบ ชุ่มชื่น ผู้เป็นน้า ซึ่งให้กำลังใจหลานอย่างดียิ่งเสมอมา

ขอบคุณ คุณ สุภัทรา สิทธิวรรณศักดิ์ ผู้เป็นที่รัก เป็นสายลมที่อบอุ่น ทำหน้าที่คู่ชีวิตได้อย่างดีเยี่ยมตลอดระยะเวลาที่ข้าพเจ้ากำลังศึกษาต่อ

ขอบคุณ ดวงตาทุกดวง สัตว์ทุกตัว หนังสือในห้องสมุดทุกเล่ม เพื่อนร่วมงานทุกคน ที่ทำให้ข้าพเจ้าได้เรียนรู้ เก็บเกี่ยวประสบการณ์ จนเป็นหมอที่ดีในวันนี้

I Content

1. Introduction

Gram-positive bacteria especially *Staphylococcus* spp. have been shown to be the most common isolates as physiologic microbiota of animal's ocular surface, which includes the eyelid, conjunctival sac, and cornea (1-4). Less commonly found gram-positive bacteria include strains such as *Streptococcus* spp., *Corynebacterium* spp., and *Bacillus* spp. (1-3). When considering gram-negative bacteria on the ocular surface of clinically healthy animals, *Moraxella* spp, and *Pseudomonas* spp. have been proven to be the most common bacteria (2, 3).

Most articles agree that the difference in the frequency and the type of bacteria in the various studies reflects the variation of the normal flora, because of the influence of geographic location, climate, season, breed of animals, nutrition, the anatomic region, and differences of culture technique (4-7). Prado et al. and Wang et al.'s studies compared the conjunctival flora in dogs without eye disease with that of dogs with ulcerative keratitis (5, 7). Both found that *Staphylococcus* spp. was the most frequently isolated genus and the group of dogs with corneal ulcers had a higher number of colony-forming units compared to the group of dogs with healthy eyes (5, 7). They also found that dogs with ulcerative keratitis had a higher percentage of the pathogenic gram-negative bacteria such as *Pseudomonas aeruginosa* (5, 7). Moreover, the results of these two studies are similar to those of Gerding et al., who studied pathogenic bacteria and fungi associated with external ocular diseases in dogs (8).

Intraoperative and postoperative ocular infections are a daunting challenge for ophthalmologists. These infections can be caused by bacteria, fungi, viruses, and protozoa, and they can be transmitted from multiple sources on the patients, including conjunctiva, eyelids, and noses (9-14). In addition, numerous external sources of contamination during ophthalmic surgery have been identified, including contaminated surgical fluids, surgical equipment, environmental factors, and surgical techniques (14-16). Since the ocular tissue of the patients

is injured and exposed to the environment during the surgical process, microbial organisms may penetrate the wound, with conjunctival bacteria being especially likely to be the source of infections during the intraoperative and postoperative period (17). In an attempt to reduce the risk of infection, many different operative and post-operative techniques have been explored, including disinfection of the surgical field and the use of a topical antibiotic (18-22). The disinfection regime can be applied prior to, simultaneously with or topical antibiotics after ophthalmic surgery are given (23-28).

Povidone-Iodine (PI) is the substance that has been proven to reduce the risk of ocular infection after eye surgery (22). In previous times, before the use of PI, a mild silver protein was commonly used to sterilize the surgical site (29). However, this practice was discontinued in 1983 after one study showed that mild silver protein had a minor bactericidal effect (21). In combination with these findings, a study from Speaker et al. reported that the preoperative application of PI had a significantly lower incidence of endophthalmitis compared with the eyes applied with mild silver protein solution (30). Thereafter, PI quickly replaced other sterilization agents for animals undergoing ocular surgery and became the most popularly used sterilization agent in preparing the surgical field (21, 30). PI is a broad spectrum disinfectant as it effectively kills *in vitro* bacteria, fungi, viruses and protozoa (31).

The bactericidal activity of diluted PI has been demonstrated in several *in vitro* studies (32, 33). This activity is the result of released free iodine from the polyvinylpyrrolidone-complex and the cytotoxicity of the released product in the cell membrane causing bacterial cell death (34). One *in vitro* study has indicated that diluted PI has a more significant bactericidal effect than 10% PI stock solution, because dilution of PI results in a weakening of the iodine linkage and increases the amount of free iodine available (33). The brown color of the solution is very easy to identify in the tear film and makes the confirmation of proper installation on the ocular surface relatively unproblematic (26). The compound is not only

available in both solution and powder form, making it ideal for transportation and storage, but it is also an inexpensive compound, making its application practicable and affordable (26). Moreover, no ocular irritation is caused when PI is diluted below 1% (25, 27, 35). However, PI can be toxic to the eye when used in higher concentrations such as 5%. Corneal edema (25, 35), conjunctival discomfort (24) and eye irritation (27) can occur after treatment with these higher concentrations.

Many studies demonstrated that the use of antibiotic eye drops both before the and during surgery reduced the incidence of endophthalmitis more effectively than in patients undergoing ocular surgery without the use antibiotic eye drops (23, 36, 37). Isenberg et al. have also compared the antibacterial effects of PI and Neosporin eye drop administration prior to surgery (19). They found that each regimen caused a similar decrease in the number of colonies and species cultured (19). However, the administration of antibiotics to animals must be carefully monitored in order to prevent antibiotic resistance due to the fact that a high occurrence of multidrug resistance in animals has been sporadically found in many countries of the world. As a result of the sporadic and often unpredictable nature of the of the problem, there should nonetheless be a concerted global effort to reduce the use of antibiotics in animals.

Therefore, the study with title *Evaluation of the effect of Povidone-Iodine (PI) on the ocular surface bacteria and determination of the antimicrobial activity of 0.2% PI in dogs and cats undergoing ophthalmic surgery* was conducted. The aim of the study was to identify the most effective PI dilution, while inducing the least side effects on the ocular surface of dog and cat and the assessment of 0.2% PI antimicrobial activity. The advantages of the study were to analyze the effectiveness of various PI concentrations on microbial organisms on the ocular surface of small animals with eye disease. By conducting a study examining variable levels of PI application, the study should yield an ideal balance between effective treatment and patients comfort. This information might improve our knowledge of ocular surface disinfection before

eye surgery and should be of interest to readers in the areas of small animal surgery and veterinarian practitioners.

A concurrent study was conducted, in equine patients undergoing ocular surgery. A high rate 6/46 (13%) of Methicillin-Resistant *Staphylococcus aureus* (MRSA) on the ocular surface of the horses was accidentally found. Therefore, further investigation was conducted to understand the genetic relationship and the origin of MRSA.

Within the past fifty years, MRSA has emerged as a major problem in horses and other companion animals around the world (38-68). Hence, MRSA has become a serious problem not only for humans but also for animals and deserves scientific attention. Unfortunately, literature regarding the occurrence and characteristics of antibiotic-resistant bacteria obtained from the ocular surfaces of horses in Europe is scarce. Therefore, the study with title *Occurrence and molecular composition of Methicillin-resistant Staphylococcus aureus isolated from ocular surfaces of horses presented with ophthalmologic disease prior invasive procedures* was conducted. This study focused on the phylogenetic relationship and molecular characteristics of MRSA isolated from equine ocular surfaces prior to invasive ophthalmological manipulation.

Staphylococcus sp. is classified as an opportunistic pathogen, which causes no problem under normal conditions (65). If an animal gets injured for another reason, this bacterium can take advantage of the weakened immune system, most commonly causing infections in the skin and soft tissue (39, 69). The most potent pathogenic staphylococci are coagulase positive, including *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus pseudintermedius*, which have been widely studied in veterinary research (70-72). Although *S. aureus* organize in a group of bacterial flora on the ocular surface of the animal, some strains can develop into potential pathogens by expressing genes called *mecA* and *mecC*, which can affect penicillin-

binding proteins, rendering the use of ß-lactam antimicrobial therapy ineffective (Figure 1) (38, 68, 73-75).

MRSA can be detected by susceptibility testing or by using PCR to confirm the presence of *mecC* or *mecA* genes responsible for methicillin resistance (76). In testing, it is crucial to determine the type of MRSA present in order to investigate the correlation between different isolates, to check whether they are of the same type, similar, or originate from the same source, and determine possible routes of transmission in the animal population (76). The genotyping methods most commonly used are Multi-Locus Sequence Typing (MLST) analysis, sequencing the protein A gene region of *S. aureus* (*spa* typing), and Pulsed–Field Gel Electrophoresis (PFGE) (76).

Antibiotic resistance is an increasing concern worldwide, especially due to the rise in nosocomial infections (77, 78). Multidrug-resistant bacteria such as methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli*, multidrug-resistant *Salmonella* serovars (MDR), and MRSA call for control strategies for veterinary patients and healthcare personnel (79).

MRSA strains are not just resistant to methicillin, but also to all antibiotics in the same drug family such as penicillins, cephalosporins, and carbapenems (80). This may lead to therapeutic problems, for instance when a colonized individual needs to receive invasive manipulation and develops an infection (79, 81). More recently, MRSA has been widely investigated in veterinary medicine, both in companion and in livestock animals (77, 82). One study from England discovered that dogs, cats, and horses undergoing hospital treatment carried MRSA more frequently than healthy animals (83). Interestingly, the initial report on MRSA infection indicated that horses are more susceptible to sporadic infection, contracted from veterinary staff (84). The reason is that microflora are closely linked and MRSA can be

transmitted from humans to horses and vice versa (84). Therefore, the incidence of MRSA colonization is higher in horses leading to an increase in the number of nosocomial infections in veterinary staff at the veterinary hospital (84).

MRSA can be spread in hospital environments via personnel and other means such as direct contact with colonized, infected individuals or indirect contact with other animals through contaminated surfaces like feed bins, water bowls, and fence rails (38, 64, 68). MRSA colonization can occur on the skin, nasal area, and soft tissues of animals and various studies suggest the nasal area as the primary source of colonization because it is more commonly in contact with the hospital environment such as the stall wall, hay, feed bin, and water bucket (38, 47, 50, 62, 65, 85-87). Many studies have also found that MRSA can be appear in animals due to prolonged administration of antibiotics (14, 38, 68, 75). Additionally, animals that have contact with health care personnel dealing directly with patient animals and especially those animals that visit the hospitals several times or stay there for extended periods are more likely to acquire multi-resistant bacteria (5, 38, 46, 68). Meanwhile, other studies have shown that bacterial infection in animals such as horses outside of clinical environments seems to be rare (44, 88, 89).

With a rate up to 41.3%, high MRSA isolation rates were found in horses in treatment at the veterinary hospital and outside the clinical environment in places such as farms (Table 1). In comparison, MRSA in dogs and cats is found in a smaller percentage. Morgan et al. reported that dogs could be infected or colonized with MRSA at a higher rate than other small animals such as cats (85).

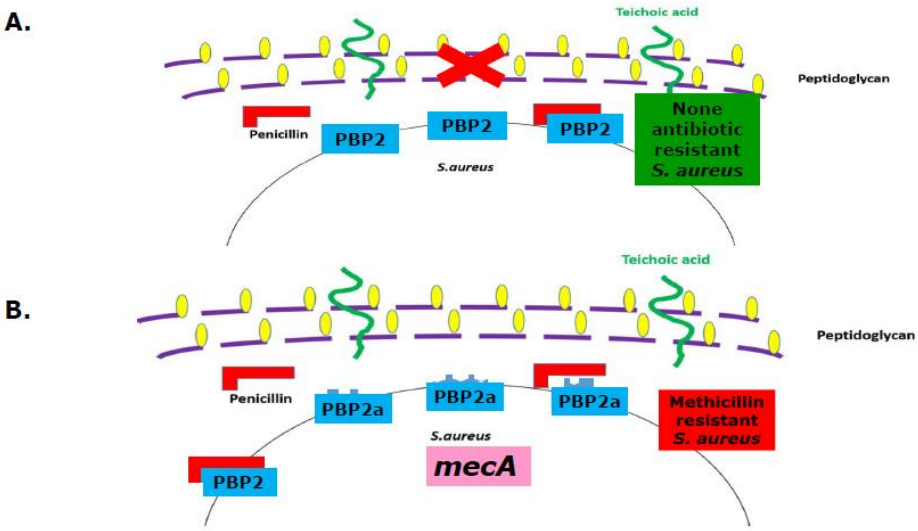

Figure 1: Resistant mechanism of Methicillin-resistant *Staphylococcus aureus*

A = Penicillin inhibit peptidoglycan synthesis by binding to penicillin binding proteins (PBP2)

B = Peptidoglycan is still synthesized by PBP2a because *mecA* gene changes the surface of the penicillin-binding protein (PBP2a) and then Penicillin cannot bind with PBP2a.

Table 1: Prevalence of MRSA from various sample sites from horses in the last 10 years (41, 44, 54, 63, 64, 66, 67, 88-90)

Population	Sample site	Country	Prevalence	Reference
HA	Nose	Cannada	2.9%	Weese et al. (2006)
HA	Nose	Belgium	10.9%	Van den Eede et al. (2009)
HEH	Nose	UK	12.0%	Baptiste et al. (2005)
HA	Wound	Germany	41.3%	Vincze et al. (2014)
HA	Nose	Ireland	5.2%	Abbott et al. (2016)
HC	Nose, mouth, axilla, perianal area	UK	2.0%	Loeffler et al. (2011)
HF	Nose	USA and Canada	4.7%	Weese et al. (2005)
HF	Nose	UK	0.0%	Baptiste et al. (2005)
HF	Nose	Slovenia	0.0%	Vengust et al. (2006)
HF	Nose and Pastern	Netherland	0.0%	Busscher et al. (2006)
HF	Nose	USA and Canada	0.7%	Anderson et al. (2007)

HA: Horse in hospital admissions, HEH: Horse stationary in equine hospital, HC: Horse in the community, HF: Horse in farm

Table 2: Prevalence of MRSA from various sample sites from the small animal last 10 years (41, 44-46, 49-51, 53, 55, 56, 58, 88, 91, 92)

Population	Sample site	Country	Prevalence	Reference
DC	Nose	UK	0.0%	Baptiste et al. (2005)
DC	Nose	Slovenia	0.0%	Vengust et al. (2006)
DC	Nose	China	0.7%	Boost et al. (2007)
DCC	Nose	USA	4.0%	Kottler et al. (2008)
DC	Skin	USA	0.0%	Griffelth et al. (2008)
DCC	Nose, mouth, axilla, perianal	UK	1.8%	Loeffler et al. (2010)
DCC	Nose and perianal	USA	1.0%	Gingrich et.al. (2011)
DCC	Nose and rectum	USA	1.3%	Bender et al. (2012)
DC	Conjunctiva	USA	0.0%	Mouney et al. (2015)
DVA	Nose	Denmark	0.0%	Bagcigil et al. (2007)
DVA	Nose, axilla and rectum	Cannada	0.5%	Hanselman et al. (2007)
DVA	Cornea and Nose	USA	1.3%	Lopinto et al. (2015)
DCVA	Nose	Ireland	1.1%	Abbott et al. (2016)

DC: Dog in the community, DCC: Dog and cat in the community, DVA: Dog in veterinary hospital admissions; DCVA: Dog and cat in veterinary hospital admission

2. Research publications in journal with peer-review

2.1. Evaluation of the effect of Povidone-Iodine (PI) on the ocular surface bacteria and determination antimicrobial activity of 0.2% PI in dogs and cats undergoing ophthalmic surgery.

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Abstract

Objective This study investigated the disinfection effectiveness and tissue irritation of varying concentrations of Povidone-Iodine (PI). Subsequently, the most effective and harmless concentration was used to assess antimicrobial activity on the ocular surface of small animals undergoing ophthalmic surgery.

Animals studied Part1: Forty eyes: (24 canine and 16 feline) were divided into four groups of ten according to the concentrations of PI 0.1%, 0.2%, 1% and 5%. Part 2: Seventy-four eyes (48 dogs and 8 cats) were included for assessment of the antimicrobial activity of 0.2% PI.

Procedure In both studies, ocular swabs were collected before aseptic preparation (t0), after aseptic preparation (t1) and after surgery (t2). Tissue reaction was monitored at t1 in part 1. Specimens were analyzed for culturable aerobic and anaerobic bacteria and fungi. The prevalence of positive culture swabs was used to measure the effectiveness of PI.

Results Part 1: The most efficient concentration of PI for microbial decontamination was 0.2%. Eye irritation was detected only in the 5% PI group. Part 2: Positive cultures were obtained from 66/74 (89%) swabs at t0, in 38/74 (51%) swabs at t1, and in 43/74 (58%) swabs at t2. The number of positive cultures was reduced at t1 and t2 compared to t0 ($p<0.0001$), but not between t1 and t2 ($p=0.859$). With the use of 0.2% PI, *Bacillus* sp. and coagulase-negative *Staphylococcus* sp. were significantly reduced at t1 and t2 ($p<0.05$).

Conclusion The most efficient concentration of PI was proven to be 0.2%. It demonstrated excellent antibacterial activity against *Bacillus* sp. and coagulase-negative *Staphylococcus* sp.

Key words: conjunctiva, cornea, dilution, disinfectant, eye**,** polyvinylpyrrolidone iodine

Introduction

Postoperative ocular infection is a challenging situation for ophthalmologists, as it can be caused by bacteria, fungi, viruses and protozoa (9-12). The incidence varied from 0.05% to 0.13% in humans (93, 94). Additionally, this problem can have catastrophic consequences including the loss of an eye (18). Several studies reported that the conjunctiva, eyelid, and nose are the primary sources of bacterial contamination causing infection (9, 13). Thus, attempts are being made to reduce this risk, in particular by using topical antibiotics and sterile surgical technique (18-22).

Antibiotic drops and proper disinfection of the surgical area with Povidone Iodine (PI) are widely used in clinical practice and have been proven to reduce the risk of ocular infection following ophthalmic surgery (23, 25, 36, 37). Many studies demonstrated that the use of antibiotic eye drops before or during an ophthalmic procedure can reduce the incidence of endophthalmitis (23, 36, 37). One study indicated that Neosporin eye drops and PI administration before ophthalmic surgery caused similar decreases in the number of colonies and species cultured (19). However, the usage of an antibiotic has to be carefully monitored to prevent antibiotic resistance (18). PI can be offered as an alternative to antibiotics (26).

PI was discovered in 1981 by Bernard Courtois and has been recognized as a valuable disinfectant for more than a decade (19, 22, 37, 95). This solution was widely used in the aseptic preparation of the eyelid and conjunctival sac before ophthalmic surgery (96). It has been described that no ocular irritation is caused when PI is diluted below 1% (25, 27, 35). It is inexpensive and has a broad antimicrobial spectrum, which makes it an ideal solution (97). The antimicrobial spectrum of PI is very comprehensive and effective in killing bacteria, fungi, viruses and protozoa *in vitro* (31, 34).

The bactericidal activity of diluted PI has been demonstrated in several clinical studies (32, 33). This activity is the result of released free iodine from the polyvinylpyrrolidone-complex and the cytotoxicity of the released product in the cell membrane causing bacterial cell death (34). Ferguson et al. reported that after the ocular surface was scrubbed using 5% PI, the bacterial kill rate was 96.7% after 60 seconds (32). Additionally, Ruth et al. (33) discovered *in vitro* that *Staphylococcus aureus* and *Mycobacterium chelonei* could not survive 15 seconds after exposure to 1% PI or 60 seconds after exposure to 0.1% of PI. One *in vitro* study has indicated that diluted PI has a more significant bactericidal effect than 10% PI stock solution (33). Dilution of PI results in a weakening of the iodine linkage and increases the amount of free iodine available (33). In a study conducted by Roberts et al., the antibacterial activity of PI 0.1%, 0.2%, 1%, and 5% on the ocular surface in dogs with healthy eyes was evaluated (25). They recommended 0.2% PI as the ocular surface disinfectant in dogs (25).

Nevertheless, there is a lack of studies on the effect of PI on the ocular surface of small animals with eye disease regarding disinfection, tissue irritation and recontamination during surgery. Therefore, this study aimed to investigate different concentrations of PI for tissue irritation and effectiveness in the elimination of microbial organisms. Subsequently, the most effective and harmless concentration was used to assess antimicrobial activity on the ocular surface of small animals with ocular lesions.

Materials and Methods

Animals

Part 1: To investigate the effectiveness of varying concentrations of PI, small animals undergoing enucleation were included. Prospectively, animals were randomly divided into four groups of ten each, according to the concentration of PI, 0.1%, 0.2%, 1% and 5%. Part 2: Small animals undergoing ocular surgery were included as additional controls to assess the

antimicrobial activity on the ocular surface of 0.2% PI. Any ophthalmic surgeries were accepted.

Questionnaires and Medical Records

Data from medical records including the use of any antibiotics (systemic and topical) in the previous six weeks were collected. Additionally, owner questionnaire interviews were conducted to gather information concerning housing maintenance and the presence of and contact with other animals.

Disinfectant

A **s**tock solution of PI 10% (BRAUN, Melsungen, Germany) was diluted with sterile 0.9% NaCl (BRAUN, Melsungen, Germany) at dilution ratios of 1:100 (0.1%), 1:50 (0.2%), 1:10 (1%) and 1:2 (5%). PI was prepared fresh and used within 24 hours to prevent microbial contamination pre-operation.

Cleansing

Three times Twenty milliliters of 0.9% NaCl was used to flush the ocular surface (eyelid, conjunctival sac, third eyelid and cornea). A von Graefe forceps was used to grab and retract the third eyelid to allow flushing of the cornea and conjunctival sac. After that, the front and back of the third eyelid were also flushed with NaCl. Then, the eyelid and surgical area were scrubbed three times using PI with gauze (KARL BEESE GmbH, Barsbüttel, Germany) approximately 3 centimeters around the eyelid margin. The estimated contact time of PI and the ocular surrounding was 2 minutes. Ocular tissue reaction was observed immediately after aseptic preparation and graded as shown in Table 1.

Sample collection

Five to ten minutes before aseptic preparation, all animals received a single injection of an antibiotic (Amoxycillin - Clavulanic acid: 12.5 mg/kg or Cefazolin 20-25 mg/kg). Sterile swabs (HEINZ HERENZ, Hamburg, Germany) were moistened with sterile water (BRAUN, Melsungen, Germany) and were obtained from the ocular surface at pre-aseptic preparation (t0), after aseptic preparation (t1), and after surgery (t2) without topical anesthetic. Sampled areas included the dorsal and ventral conjunctival fornix and the eyelid margin. After sample collection, specimens were immediately submitted to the laboratory.

Microbiological examination

All swabs were examined for culturable aerobic bacteria, anaerobic bacteria and fungi at the Institute of Microbiology and Epizootics, Faculty of Veterinary Medicine, Freie Universität Berlin, Germany. Within 12 hours of collection, swab samples were stored at 4°C until processing. The swabs were analyzed by aerobic and anaerobic cultivation after direct inoculation on suitable agar plates (all agar purchased from Oxoid, Wesel, Germany). For the detection of aerobic bacteria, Columbia blood agar (5% sheep blood), Gassner agar and Brilliance UTI Clarity agar were inoculated within 12 hours after sampling for 24-48 hours (aerobic, 36°C). For detection of fungi Sabouraud Dextrose agar was inoculated (aerobic, 28° C for 96 h). For detection of obligate anaerobic bacteria, the inoculation was carried out on Columbia blood agar (5% sheep blood) with added L-cysteine (Merck), haemin (Sigma-Aldrich, Hamburg, Germany), vitamin K1 (Roche, Mannheim, Germany) and lysed sheep blood 0.5% (Oxoid) for 48-72 hours (anaerobic, 36°C). Chocolate agar was used for cultivation of fastidious bacteria for 24-48 hours (36°C, microaerobic, 7% CO2). Species identification was performed by colony morphology evaluation, Gram stain and catalase test according to standard protocols (Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology. Washington DC, American Society of

Microbiology, 2015.) and MALDI-TOF MS (Bruker Microflex LT, Biotyper 3.0 software; Bruker Daltonics, Bremen, Germany). Any bacteria or fungi isolated from a swab was considered to be a positive culture. Microbial range was defined as the alteration of the spectrum of microbial organisms at t1 or t2.

Statistics

Descriptive statistics were used to assess the validity of the data. A normality test (Kolmogorov-Smirnov or Shapiro-Wilk test) was used to determine the distribution of data. Cochran's Q test was used to determine the effectiveness of PI using positive cultures at each time point (t0, t1, and t2). A chi-square test or Fisher's exact test was used to examine the association between specific factors and positive cultures (breed, sex, age, ocular side, part of the disease, housing, the presence of and contact with other animals, the long-term duration of systemic/topical antibiotic treatment). Logistic regression was used to examine the association between ratio scales (age) and positive cultures. The data were analyzed using SPSS for Windows version 22, and a p-value < 0.05 was considered significant.

Results

Part 1

To investigate the effectiveness of varying concentrations of PI**,** 16 cats and 24 dogs undergoing enucleation were included. The group of patients consisted of 17 males and 23 females. The mean age was 90 months (min-max = 7-213 months, median = 81 months) The breeds of small animals were 25.0% (10/40) mixed breed and 75.0% (30/40) pure breed. They were diagnosed with 40.0% (16/40) infected corneal disease and 60% (24/40) intraocular disease (uveitis, glaucoma, cataract and lens luxation).

Out of 120 samples, 75 (62.5%) demonstrated positive culture result, at t0, 38/40 samples were positive culture (95%); at t1, 18/40 samples (45%) were positive; and at t2, 19/40 samples

(47%) were positive culture. Generally, positive cultures were reduced at t1 and t2 in comparison to t0 at PI concentrations of 0.2% (p=0.002), 1% (p=0.006) and 5% (p=0.004), but not at a PI concentration of 0.1% (p=0.368), as shown in Table 2.

In detail (Table 2), positive cultures from t1 were significantly reduced when compared to t0 (p<0.05) after the use of 0.2%, 1% and 5% PI. At t2, the eyes disinfected with 0.2% and 1% PI showed a significant decrease in positive cultures when compared to t0 (p<0.05). After ocular surfaces were disinfected with 5% PI, there was no significant reduction of microbial contamination in t2 in comparison to t0 (p>0.05).

After disinfecting with 0.1%, 0.2%, and 1% PI, the ocular surfaces showed no sign of tissue reaction. However, when 5% PI was used, chemosis of the conjunctiva was found in 1 dog. Two dogs showed periocular redness and swelling. After 10 seconds, one cat and one dog disinfected with 5% PI showed corneal erosion. Nine patients (3 dogs and 6 cats) revealed redness of the conjunctiva and the third eyelid. Increased injected episcleral blood vessels were found in the ocular tissue of every patient after contact with 5% PI.

Part 2

To assess the antimicrobial activity of 0.2% PI on the ocular surface, 56 small animals (48 dogs and 8 cats; n=74 eyes) undergoing ophthalmic surgery were included.

The mean age was 53 months (range = 0-176 months, median = 46 months). The breeds of the small animals were 16.0% (9/56) mixed breed and 84.0% (47/56) pure breed. Their lesions were 46.3% (34/74) conjunctival and eyelid disease, 24.0% (18/74) infected corneal disease, 23.0% (17/74) intraocular disease (uveitis, glaucoma, cataract and lens luxation) and 6.7% (5/74) other eye diseases.

The type of surgery was 6.7% (5/74) eyelid tumor excision, 12.1% (9/74) distichiasis excision, 27.0% (20/74) eyelid correction, 8.1% (6/74) pocket technique, 5.4% (4/74) conjunctival flap,

5.4% (4/74) keratectomy, 18.9% (14/74) extracapsular lens extraction, 16.2% (12/74) other surgeries.

In total, 222 swabs were collected, and in 66% (147/222) of bacteria were isolated. A single isolate was obtained from 46% (67/147), two or more species were identified in 49% (73/147), and unspecific species of the microbial organism were isolated in 5% (7/147).

A total of 222 microbial isolates were obtained from 222 swabs (Table3) The most frequently recovered gram-positive bacterial specie was *S. pseudintermedius*, at 26.1% (58/222). *Enterobacteriaceae* spp. were the predominantly isolated gram-negative bacteria, at 8.1% (18/222). The majority of the isolated yeast were *Candida albicans* 1.3% (3/222).

At t0, 66/74 (89%) swabs were culture positive, whereas only 38/74 (51%) of the samples collected at t1 and 43/74 (58%) of the samples at t2 yielded positive results (Figure 1). Thus, the number of positive cultures was significantly reduced at t1 and t2 compared to t0 (p<0.0001). No significant differences were detected between t1 and t2 (p=0.859).

After the ocular surface was disinfected with 0.2% PI, the number of microbial organisms decreased to 92% (68/74) at t1 and to 84% (62/74) at t2 (p>0.05), and the microbial range was overall comparable at 90% (67/74) at t1 and 95% (70/74) at t2, in comparison to t0 (p>0.05).

The results concerning the antibacterial activity of 0.2% PI as a disinfectant on the ocular surface of small animals showed that *Bacillus* sp. and coagulase-negative *Staphylococcus* sp. were significantly reduced at t1 and t2 (p<0.02 and <0.001, respectively), while *Staphylococcus pseudintermedius* were significantly reduced only at t2 (p=0.034).

Patients showing lesions on the ocular surface (eyelid or conjunctiva) had a significantly higher chance for positive microbial culture at t1 and t2 (p<0.05) than animals that had corneal or intraocular lesions (p>0.05). Breed, sex, age, ocular side, housing, the presence of and contact with other animals and the long-term duration (more than 4 weeks) of systemic/topical

antibiotic treatment had no significant influence on the presence of positive microbial cultures (p>0.05).

Discussion

The most efficient dilution for the elimination of microbial organisms was 0.2% PI, while 0.1% PI was the least effective. Statistically significant changes could not be detected from the eyes disinfected with 0.1% PI. After treatment of the eyes with PI 0.1%, 0.2% and 1%, no signs of ocular tissue irritation were noticed. Only after the use of 5% PI, ocular tissue irritation was detected. After confirming that 0.2% PI was the most effective and least harmful concentration, an additional survey of the antimicrobial activity of 0.2% PI (part 2) was conducted. The number of positive cultures was significantly reduced at t1 and t2 compared to t0, but no significant differences between t1 and t2 could be detected. Gram-positive bacteria such as coagulase-negative *Staphylococcus* spp. were predominantly isolated at t0. Additionally, the results showed that *Bacillus* sp. and coagulase-negative *Staphylococcus* spp. were significantly reduced at t1 and t2 (p<0.05).

When compared to various concentrations in our study (Part 1), 0.2% PI had the most significant reduction in positive cultures (p=0.02), compared to 0.1% PI (p=0.368), 1% PI (p = 0.06) and 5% PI (p=0.04). Our outcome is consistent with the results of a previous study conducted by Robert et al. (1986), who found that 0.2% PI was the most potent for disinfection on the ocular surface of dogs with healthy eyes (25). The 0.1% PI concentration in our study removed microbes in 3 of 10 eyes; the statistical analysis showed no significant difference in reduction of positive cultures after aseptic preparation (p=0.368). Our outcome can be supported by Robert et al. (1986), who found that 0.1% PI should not be used for ocular disinfection because bacteria such as *Escherichia coli* were found (25). The contrast in the effectiveness of diluted PI can be supported by the *in vitro* study conducted by Ruth et al. (1982), who found that the diluted PI had a more significant bactericidal effect than 10% PI

stock solution (33). Ruth et al. (1982) reported an increased bactericidal action *in vitro* as the degree of dilution increased (33). The low PI concentrations between 0.1% and 1% had a higher bactericidal activity when compared to 10% PI stock solution (33). This was due to the increase in non-complex iodine as the dilution increased (33). However, once the dilution became less than 0.1%, free iodine decreased and less bactericidal effect was observed (33). The enhancement of the bactericidal activity of diluted PI was also reported in another *in vitro* study evaluating the morphological changes in *Escherichia coli* under a microscope after treatment with PI solution (98).

Redness of the conjunctiva and third eyelid, chemosis of the conjunctiva, periocular redness and swelling, corneal erosion and increased episcleral injected blood vessels were related to exposure to 5% PI. These signs of eye irritation were noticed not only in our study but also in other clinical studies (24, 25, 27, 35). According to the study from Robert et al. (1986), corneal epithelial edema was reported in dogs after the ocular surface was disinfected with 5% PI (25). Jiang et al. (2009) found severe corneal epithelial damage after applying 5% PI into the conjunctival sac of rabbits (35). Moreover, two studies in human ophthalmology reported that the patients had ocular discomfort after the eyes contacted 5% PI (24, 27). The diluted 1% PI could have been a solution for preventing physical damage to the eyes. However, Jiang et al. (2009) found mild corneal edema after applying 1% PI into the conjunctival sac of rabbits (35). Additionally, Naor et al. (2001) reported corneal edema and endothelial cell loss after injection of 1% PI into the anterior chamber (99). In conclusion, the results of these studies and our study indicated that both 5% and 1% PI exerted detrimental effects on the ocular surfaces of small animals (24, 25, 27, 35, 99). PI in lower concentrations such as 0.2% was less toxic and more efficient at preparing the ocular surface. Therefore, 0.2% PI can be applicable for decontamination before ophthalmic surgery in small animals.

According to the statistical results in our study (Table 2), positive microbial cultures were identical at t2 (5/10) after the eye was disinfected with 1% and 5% PI, but the statistical analysis result was different (p=0.037 for 1% PI and p=0.062 for 5% PI). It is assumed that the small sample size (10 animals per group) has led to imprecise statiscal analysis.

In addition, the bacteria were shown to be similar at t0 between healthy eyes (2, 100) and diseased eyes of small animals (101, 102). The predominant bacteria isolated were gram-positive bacteria. Statistical analysis indicated that 0.2% PI was significantly effective at reducing ocular surface colonization of *Bacillus* sp. (p=0.02) and coagulase-negative *Staphylococcus* spp. ($p<0.001$) at t1 and t2 in comparison to t0, while *Staphylococcus pseudintermedius* were significantly reduced only at t2 (p=0.034) compared to t0. No significant difference in the reduction of other microbial organisms was found in our study. However, this did not indicate that 0.2% PI was less effective in the elimination of these microbial organisms. Because the numbers of some isolated microbial organisms were inadequate, the statistical result was insignificant. An investigation by Ruth et al. (1982) assessed the effect of dilute preparations of PI on the survival of bacteria after a short exposure time *in vitro* (33). They reported that *Staphylococcus aureus* and *Mycobacterium chelonei* were killed after exposure to 0.2% PI for 15 seconds and 1 minute, respectively (33).

After the eyes were disinfected with 0.2% PI, the number of microbial organisms increased in 6/74 swabs (8%) at t1 and 12/74 swabs (12%) at t2 in comparison to t0. The change in microbial range was found in 7/74 (10%) of samples at t1 and 4/74 (5%) at t2 compared to t0. Many studies reported numerous sources of contamination during ophthalmic surgery such as contaminated surgical fluids, surgical equipment, airborne organisms and surgical techniques (14-16). Our microbial contamination at t1 and t2 may come from these factors. The primary source of postoperative infection may be the bacteria residing on the eyelid and the conjunctival flora of small animals, especially gram-positive bacteria.

The study conducted by Robert et al. reported that after the eyes were disinfected with 0.2%, 1% and 5% PI, no microbial growth was found in any samples except in one eye disinfected with 0.1% PI. In our study, 51% (38/74) of sample swabs from t1 were culture positive. This phenomenon can be explained by reports from Prado et al. and Wang et al. (5, 7) on the conjunctival flora in dogs without eye disease and dogs with ulcerative keratitis (5, 7). These reports found that dogs with corneal ulcers had a higher number of colony forming units when compared to healthy dogs (5, 7). Collectively from the various studies, it can be concluded that the quantity of bacteria on the ocular surfaces of animals with eye disease can be different from animals with healthy eyes (5, 7, 8). Thus, the effectiveness of 0.2% PI in small animals with healthy eyes and small animals with eye disease can be different.

Our results concerning the efficiency of various concentrations of PI as a disinfectant on the ocular surface in cats and dogs were comparable to those from recent studies; the reduction of microbial organisms was good but not perfect (25). Therefore, aseptic preparation procedures need to be improved in general. According to a study in human ophthalmology, a significant reduction in the mean number of bacteria was achieved by using 5% PI along with Neosporin topical treatment for three days preoperatively (19). Additionally, Polyhexanide has been described as another well-tolerated antiseptic agent, which can reduce bacterial growth more efficiently than 1.25% PI (103). Chlorhexidine solution is also an option that can be used in the routine preparation of eyes for ophthalmic surgery (37). Barkana et al. (2005) reported that there was no difference in effectiveness at reducing bacterial growth between 4% PI and 0.05% Chlorhexidine in aseptic preparation for ophthalmic surgery (37). Therefore, chlorhexidine might be used as an alternative option for patients with hypersensitivity to PI. Further studies could focus on the comparison of 0.2% PI with other disinfectants such as polyhexanide and chlohexidine in terms of the effectiveness and irritation on the ocular surface of dogs and cats.

It should be the aim of ophthalmologists to find an appropriate aseptic agent that acts rapidly, attacks a broad range of microbial organisms and causes low ocular irritation.

There are several limitations in our study. First, the microbiological examination was conducted by semi-quantitative bacterial examination and qualitative data (positive culture). Hence, no conclusions on the number of the colony-forming units can be drawn. Second, the number of samples was relatively low when compared to other studies in human medicine. Therefore, the statistical results are less precise.

Conclusion

PI at a concentration of 0.2% can be used to eliminate bacterial contamination without causing a tissue reaction. It is more effective than the concentrated solution and demonstrated a statistically significant reduction of microbial growth, especially of *Bacillus* sp. and coagulase-negative *Staphylococcus* sp. Therefore 0.2% PI is the recommended concentration for aseptic ophthalmic surgery preparation.

Acknowledgement

The authors would like to thank Franziska Thienel and Nina Peche for technical assistance, the Institute for Veterinary Epidemiology and Biostatistics, Freie Universität Berlin, Germany, for statistical consulting, and Prince of Songkhla University, Thailand, for Miss Soimala financial support.

Table 1: Score used for the evaluation of ocular tissue irritation based on the Modified Draize Eye Irritation Scale (27).

Redness of sclera, conjunctiva and third eyelid	
Not injected	0
Increase of vessels injected	1
Conjunctival swelling	
No swelling	0
Swelling	1
Eyelid margin and periocular swelling	
No swelling	0
Swelling	1
Eyelid margin and periocular redness	
No redness	0
Redness	1
Corneal Erosion	
No Erosion	0
Erosion	1
Episclera injected blood vessel	
Not injected	0
Increase of vessels injected	1

Table 2: Number of positive cultures from the ocular surface of enucleated patients (n=40) at each time point.

Con	t0 +, (%)	t1 +, (%)	t2 +, (%)	t0 and t1 or t2 (*p*)	t0 and t1 (*p*)	t0 and t2 (*p*)	t1 and t2 (*p*)
0.1%	8/10 (80%)	7/10 (70%)	6/10 (60%)	0.368	*p*>0.05	*p*>0.05	*p*>0.999
0.2%	10/10 (100%)	4/10 (40%)	3/10 (30%)	0.002	0.016	0.004	*p*>0.999
1%	10/10 (100%)	4/10 (40%)	5/10 (50%)	0.006	0.008	0.037	*p*>0.999
5%	10/10 (100%)	3/10 (30%)	5/10 (50%)	0.004	0.004	0.062	*p*>0.999

The results show the different effectiveness of various concentrations of PI between different time points and concentrations. Con = Concentration, t0 = before aseptic preparation, t1 = after aseptic preparation, t2 = after surgery, p = p-value

Table 3: Frequency of microbial isolates and the frequency found at the different time points from small animals (n=222 samples) undergoing assessment of the antimicrobial activity of 0.2% povidone-iodine.

List of microbial organisms	t0	t1	t2	Total
Gram-positive bacteria				
Coagulase-negative *Staphylococcus* spp.	30	3	9	42
Staphylococcus pseudintermedius	24	18	16	58
Staphylococcus pseudintermedius (Methicillin resistant)	4	2	1	7
Streptococcus canis	15	11	9	35
Other *Streptococcus* spp.	1	0	0	1
Enterococcus spp.	1	0	0	1
Bacillus spp.	12	3	6	21
Corynebacterium spp.	3	3	2	8
Other coryneform rods	4	1	0	5
Clostridium perfringens	1	0	0	1
Total (Gram-positive bacteria)	95	41	43	179
Gram-negative bacteria				
Escherichia coli	2	2	1	5
Other Enterobacteriaceae spp.	4	5	4	13
Pasteurella multocida	2	2	2	6
Other *Pasteurella* spp.	3	2	0	5
Neisseria spp.	2	0	0	2
Moraxella spp.	1	0	2	3
Bergeyella spp.	1	0	0	1
Pseudomonas aeruginosa	1	0	0	1
Total (Gram-negative bacteria)	16	11	9	36
Fungi and yeasts				
Malassezia spp.	2	0	0	2
Candida albicans	1	1	1	3
Trichosporon sp.	1	0	0	1
Other Yeast sp.	1	0	0	1
Total Fungi and yeasts	5	1	1	7
Total (All)	116	53	53	222

t0 = pre-aseptic preparation, t1 = after-aseptic preparation, t2 = after-surgery

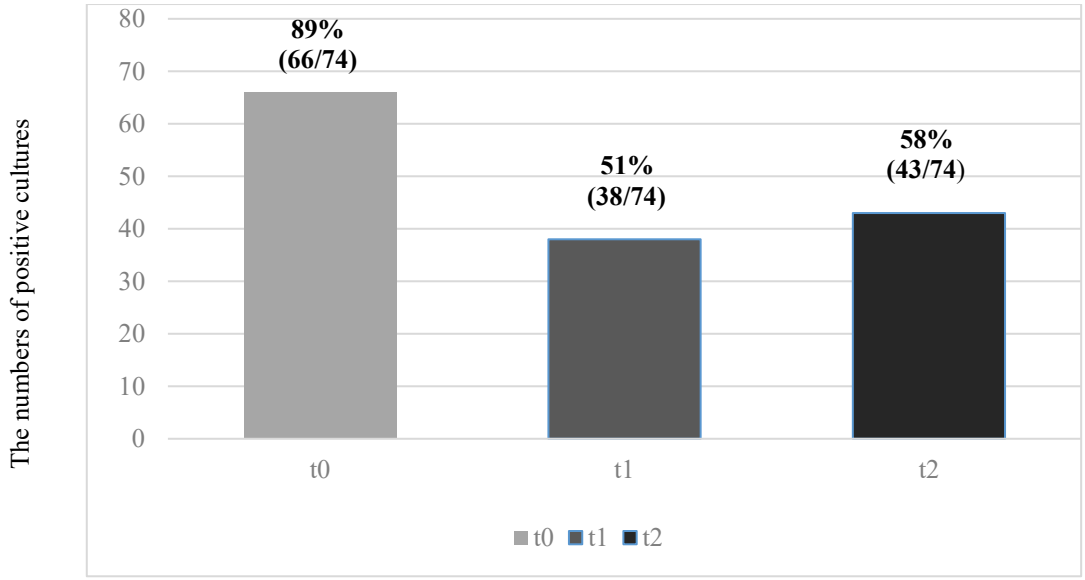

Fig 1: Positive microbial cultures from ocular surface samples obtained before aseptic preparation (t0), after aseptic preparation (t1), and after surgery (t2) from 74 eyes (n = 222 swabs) of small animals undergoing assessment of the antimicrobial activity of 0.2% Povidone Iodine.

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2.2. Occurrence and molecular composition of methicillin-resistant *Staphylococcus aureus* isolated from ocular surfaces of horses presented with ophthalmologic disease

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Veterinary Microbiology, Vol. 222, August 2018, Pages 1-6
DOI: https://doi.org/10.1016/j.vetmic.2018.06.009

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3. Discussion

The most efficient dilution for the elimination of microbial organisms was 0.2% PI, while 0.1% PI was the least effective. Statistically significant changes could not be detected from the eyes disinfected. After treatment of the eyes with PI 0.1%, 0.2%, and 1%, no signs of ocular tissue irritation were noticed. Only after the use of 5% PI was ocular tissue irritation detected. After confirming that 0.2% PI was the most effective and least harmful concentration, an additional survey of the antimicrobial activity of 0.2% PI was conducted. The number of positive cultures was significantly reduced at t1 and t2 compared to t0, but no significant differences between t1 and t2 could be detected. Gram-positive bacteria such as coagulase-negative *Staphylococcus* spp. were predominantly isolated at t0. Additionally, the results showed that *Bacillus* sp. and coagulase-negative *Staphylococcus* sp. were significantly reduced at t1 and t2 ($p<0.05$).

The results from the MRSA study showed that 6/46 (13.0%) samples were positive for MRSA in horses. MRSA-positive swabs were obtained from eyes showing clinical signs of keratitis 4/9 (44%) and uveitis 2/29 (6.9%), while none of the horses with blepharitis/conjunctivitis was found MRSA positive. Apart from resistance towards beta-lactam based antibiotics, MRSA exhibited further resistance towards antibiotics as fluoroquinolone, tetracycline, and aminoglycoside. PFGE analysis revealed a very close relatedness (>90.0%) of the six MRSA isolates, all assigned to *spa*-type t011, a *spa* type commonly associated with the European livestock-associated MRSA lineage of the clonal complex (CC) 398. The methicillin-susceptible singleton revealed *spa* type t3043 and belonged to clonal complex CC 9. According to our result, multi-resistance drug bacteria are major concern in equine veterinary ophthalmology. Therefore, the usage of an antibiotics has to be carefully monitored to prevent the antibiotic resistance.

When compared to various concentrations in our study, 0.2% PI had the most significant reduction in positive cultures (p=0.02), compared to 0.1% PI (p=0.368), 1% PI (p = 0.06) and 5% PI (p=0.04). In an *in vitro* study Ruth et al. reported an increase in the bactericidal action of PI as the degree of dilution increases (104). They found that a low concentration from 0.1% to 1% PI was more effective than the 10%PI stock solution (104). The content of non-complexed iodine relatively increased after the dilution reached a maximum at 0.1 % and decreased again for further dilution 1:10000 (0.001%) (104). By comparison, the 0.1%PI concentration eliminated bacteria in 3 of 10 eyes in our study, the statistical analysis showed no significant difference in this dilution (p = 0.368). Our result is consistent with the study from Robert et al. (25). They reported that the antibacterial activity of 0.1%PI was also relatively ineffective after exposing dog,s eyes with 0.1% PI, concluding that 0.1% PI should not be used for ocular disinfection (25).

Meanwhile, redness of the conjunctiva and third eyelid, chemosis of the conjunctiva, periocular redness and swelling, corneal erosion and increased episcleral injected blood vessels were related with 5%PI. These signs of eye irritation were noticed not only in our study but also in other clinical studies (24, 25, 27, 35). According to the study from Robert et al. (1986), corneal epithelial edema was reported in dogs after the ocular surface was disinfected with 5% PI (25). Jiang et al. (2009) found severe corneal epithelial damage after applying 5% PI into the conjunctival sac of rabbits (35). Moreover, two studies in human ophthalmology reported that the patients had ocular discomfort after the eyes contacted 5% PI (24, 27). The diluted 1% PI could have been a solution for preventing physical damage to the eyes. However, Jiang et al. (2009) found mild corneal edema after applying 1% PI into the conjunctival sac of rabbits (35). Additionally, Naor et al. (2001) reported corneal edema and endothelial cell loss after injection of 1% PI into the anterior chamber (99). In conclusion, the results of these studies and our study indicated that both 5% and 1% PI exerted detrimental effects on the ocular surfaces

of small animals (24, 25, 27, 35, 99). PI in lower concentrations such as 0.2% was less toxic and more efficient at preparing the ocular surface. Therefore, 0.2% PI can be applicable for decontamination before ophthalmic surgery in small animals.

In the evaluation of the effectiveness of PI, the bacteria were shown to be similar at t0 between healthy eyes (2, 100) and diseased eyes of small animals (101, 102). The predominant bacteria isolated were gram-positive bacteria. Statistical analysis indicated that 0.2% PI was significantly effective at reducing ocular surface colonization of *Bacillus* sp. (p=0.02) and coagulase-negative *Staphylococcus* spp. ($p<0.001$) at t1 and t2 in comparison to t0, while *Staphylococcus pseudintermedius* were significantly reduced only at t2 (p=0.034) compared to t0.

Concurent with the evaluation of PI in small animals, ocular swabs were collected from horse patients undergoing ophthalmic surgery. Our study found a high rate of MRSA 13% (6/46) on the ocular surface of the horses. By comparison with ocular swabs, nasal swabs of horses in a clinical environment showed MRSA positive-result in 10.9% in Belgium and 12% in the UK (44, 63). The isolation rate was 0% when horses were outside of a clinical environment like in the Netherlands and Slovenia (88, 89).

In our study, PFGE and *spa* type test were used to investigate the correlation between different isolates. PFGE analysis revealed a very close relatedness (>90.0%) of the six MRSA isolates, all assigned to *spa*-type t011. This *spa* type is commonly associated with the European livestock-associated MRSA lineage of the clonal complex (CC) 398, which was first reported in nasal colonization from pigs in France 2005 (105). Additionally, the pathogen was transmitted from animals to humans, therefore, it is termed a Livestock Associated MRSA (106). The reports showed that humans, who work in equine hospitals and in a livestock production environments were colonized (107-109). Five from six positive MRSA horses in

our study were sent at least two times to the hospital for ophthalmologic examination and manipulation. Another five out of six MRSA positive animals were hospitalized at least 18 hours prior sampling. This group of animals seemed to be at higher risk through contact with MRSA in the hospital and hospital personel. According to the history of equine patients and the result of the microbiological examination, it can be suggested that this phenomenon was a nosocomial infection.

Antibiotic resistance in bacteria is a serious problem and many strategies have been used to address this issue. For instance, the patients should receive a bacterial culture and drug sensitivity test before receiving antibiotic treatment (110, 111). In addition, the prescription should specify the dose, duration, and indication for every antibiotic (110, 111). The use of potent antibiotics should not be used for mild surface infection (112). Furthermore, long-term administration of topical antibiotics should only be applied insidiously (110). According to our results, MRSA is resistant towards antibiotics that are frequently used for topical eye treatment e.g. fluoroquinolone, tetracycline, and aminoglycoside. Constant bacterial surveillance is needed as ocular manipulation might be a risk factor for the equine patient.

Since MRSA was not positive in our PI evaluation, the effectiveness of 0.2% PI on eliminating MRSA on the ocular surface of animals is still an open question. According to the investigation from Ruth et al., they assessed the effect of diluted concentrations of PI *in vitro* on the survival of bacteria after a short exposure time and reported that *Staphylococcus aureus* and *Mycobacterium chelonei* were killed after exposure 0.2% PI in 15 seconds and 1 minute respectively (33). Haley et al. also reported on the bactericidal activity *in vitro* of antiseptic agents against MRSA (113). Haley et al. evaluated the stock strength and in serial 10 – fold dilutions of 3% hexachlorophene (Phisihex), 4% chlorhexidine gluconate-alcohol (Hibiclens), 1% *p* – chloro – m – xylenol (Acute- Kare) and diluted PI (Betadine) (113). PI was the most efficient at 0.1% concentration, all MRSA were killed in 15 seconds, while other dilution of PI

failed to kill all the MRSA (113). Additionally, a study in 1983 compared the efficacy of PI against MRSA between Betadine solution (10% PI), and Betadine cream (5%PI) *in vitro* (114). The results showed that PI solution (10%PI) was effective against all MRSA isolates in 30 seconds (114). While PI cream (5%) PI killed four of the MRSA isolates within 30 seconds and required 2-3 minutes killing time for one strain of MRSA (114).

Whereas our results concerning the efficiency of various concentration of PI as a disinfectant on the ocular surface of cats and dogs were comparable to those of recent studies, the reduction of the bacterial was good but not perfect (25). Therefore, aseptic preparation procedures need to be improved in general. According to a study in human ophthalmology, a significant reduction in the mean number of bacteria was achieved by using 5% PI along with Neosporin topical treatment for three days (19). Additionally, Polyhexanide has been described as another well tolerated antiseptic agent, and it was more efficient in reducing bacterial growth when compared to 1.25% PI in human ophthalmology (103). Chlorhexidine solution alone is also a choice to use for the routine preparation of eyes for ophthalmic surgery. While never tested in clinical trial, Gelett et. al. recommended that chlorhexidine 0.05% is a safe and effective antimicrobial disinfectant for the dog's cornea and conjunctiva (115). Meanwhile, Barkana et al. reported that there was no difference in the reduction of bacteria by 4% PI and 0.05% Chlorhexidine in aseptic preparation of human ophthalmic surgery (37). Hence, it can be suggested that polyhexanide and chlorhexidine can be used for patients in cases of hypersensitivity with PI.

Preventive measures can also be considered, preoperative cultures are one option that may predict the type of local flora at a time prior to surgery. If the potential pathogenic microbial organism is MRSA positive, the ophthalmologist has a chance to treat the eye before the surgery and it can reduce the infection rate both during and after the operation. However, the reliability of this method has not be definitely proven. One study showed that 35% of

patients undergoing cataract surgery had conjunctival culture results at the time of surgery that were different compared to the previous day without the application of prophylactic antimicrobial (116). Similarly, a study by Allansmith et al. found healthy volunteers who were culture positive for *S. aureus* from the lid margin were cultural-negative when recultured more than one day later (117).

There are several limitations in our study. First, the microbiological examination was conducted by semi-quantitative bacterial examination and qualitative data (positive culture). Hence, no conclusions on the number of the colony-forming units can be drawn. Second, the number of samples was relatively low when compared to other studies in human medicine. Therefore, the statistical results are less precise.

Further studies could focus on the comparison of 0.2% PI with other disinfectants such as polyhexanide and chlorhexidine in terms of the effectiveness and irritation of the ocular surface of animals. It should be the aim of ophthalmologists to find an appropriate aseptic agent that acts rapidly, attacks a broad range of microbial organisms and especially multi-resistance drug bacteria, and causes low ocular irritation.

4. Summary

Ocular infections in animals can be result in blindness. The infections are most caused by bacteria particular microbiota from the patient's conjunctiva, eyelid, and nose. When damage to ocular tissue occurs, opportunistic bacteria may invade ocular tissues causing infection that may be difficult to treat, especially if the bacteria are antibiotic resistant like MRSA. Therefore, the usage of antibiotics has to be carefully monitored to prevent antibiotic resistance and a global reduction in a use of antibiotics a practical aim. Povidone-Iodine (PI) is generally known for being effective against bacteria, fungi, viruses, and protozoa. Moreover, an ideal solution as it is inexpensive, and no irritation is caused when diluted below 1%. One study indicated that Neosporin eye drops and PI administration before ophthalmic surgery caused similar decreases in the number of colonies and species cultured. Therefore, PI can be offered as an alternative to antibiotics.

Forty eyes: (24 dogs and 16 cats) were divided into four groups of ten according to the concentrations of PI 0.1%, 0.2%, 1% and 5%. Seventy-four eyes (48 dogs and 8 cats) were included for assessment of the antimicrobial activity of 0.2% PI. Conjunctival swabs were collected from 44 horses (46 eyes) to determine the occurrence of MRSA on the ocular surface of the horse. In the first study, ocular swabs were collected before aseptic preparation (t0), after aseptic preparation (t1) and after surgery (t2). Tissue reaction was monitored at t1. Specimens were analyzed for cultivable aerobic and anaerobic bacteria and fungi. Positive culture swabs were used to measure the effectiveness of PI. In the second study, the conjunctival swabs that were collected before ophthalmic surgery were immediately processed with testing of colonies suspected to be *S. aureus* was carried out using Vitek2 system (bioMérieux, Lyon) and the presence of MRSA was verified by PCR. Genetic relatedness of all MRSA isolates was investigated by pulsed-field gel electrophoresis (PFGE) and *spa*-typing.

The result of the first study indicated that 0.2% and 1% PI solutions could be used to eliminate bacterial contamination of ocular surface tissue without tissue reaction. However, 0.2% PI proven to be more effective than more concentrated solutions (p= 0.02). Additionally, 0.2% PI concentration significantly reduced *Bacillus* sp. and coagulase negative *Staphylococcus* sp. at t1 and t2. MRSA was not positive in our first study. That is why the effectiveness of PI on eliminating MRSA on the ocular surface of animals still undetermined.

The results of the second study indicated that the isolation rates of MRSA on the ocular surface of horses were high (13%) 6/46 samples. Molecular typing revealed that all detected MRSA were the *spa*-type t011. This type is commonly associated with the European livestock-associated MRSA lineage of the clonal complex CC 398 that recently emerged in horses in Germany. Additionally, PFGE revealed that the six MRSA had a very closely related relationship (>90%). According to the history of the patients and the result of the microbiological examination, it can be suggested that this phenomenon was a nosocomial infection. MRSA reported in our study exhibited further resistance towards antibiotics frequently used for topical eye treatment, e.g. fluoroquinolones, tetracycline's, kanamycin and gentamicin.

The most effective concentration of PI was proven to be 0.2%. It demonstrated excellent antibacterial activity against *Bacillus* sp. and coagulase-negative *Staphylococcus* sp. This study demonstrated a high prevalence of MRSA on the ocular surfaces of equine ophthalmic patients supporting the need for constant bacterial surveillance.

More studies are needed on the source of MRSA in equine ophthalmic patients including an environmental factor, standard operation procedures (SOPs), manipulations and diagnostic approaches. The evaluation of the bactericidal activity of antiseptic agents against MRSA on the ocular surface of animals at either higher concentrations of PI or alternative protocols and

active components to reduce ocular surface contamination are still needed.

5. Zusammenfassung

Hygiene in der Augenchirurgie und bakterielle Resistenzen auf der Augenoberfläche bei Tieren

Augeninfektionen bei Tieren können zur Erblindung führen. Die Infektionen können durch Bakterien, insbesondere die Mikrobiota der Bindehaut, des Augenlides und der Nase des Patienten verursacht werden. Bei Beschädigung des Augengewebes, können die opportunistischen Bakterien in das Auge eindringen und zu einer Infektion führen, welche schwierig zu behandeln ist, insbesondere dann, wenn die Bakterien antibiotikaresistent sind, wie MRSA. Deshalb muss der Einsatz eines Antibiotikums sorgfältig überwacht werden, um eine Antibiotikaresistenz zu verhindern. Den Einsatz von Antibiotika zu reduzieren, ist ein globales Ziel. Povidone-Iodine (PI) ist bekannt dafür, dass es gegen Bakterien, Pilze, Viren und Protozoen wirksam ist. Darüber hinaus ist es günstig und verursacht keine Irritation, wenn es unter 1% verdünnt wird. In einer Studie konnte gezeigt werden, dass Neosporin-Augentropfen und die Verabreichung von PI vor Augenoperationen eine ähnliche Reduktion an kultivierten Bakterienkolonien und -spezies verursachten. Deshalb kann PI als Alternative zu Antibiotika genutzt werden.

Vierzig Augen: (24 Hunde und 16 Katzen) wurden in je vier Zehnergruppen entsprechend der Konzentrationen von PI 0,1%, 0,2%, 1% und 5% eingeteilt. Vierundsiebzig Augen (48 Hunde und 8 Katzen) wurden zur Beurteilung der antimikrobiellen Aktivität von 0,2% PI beurteilt. Von 44 Pferden (46 Augen) wurden Konjunktivalabstriche gesammelt, um das Auftreten von MRSA auf der Augenoberfläche des Pferdes zu bestimmen. In der PI Studie wurden Augenabstriche vor der aseptischen vorbereitung (t0), nach der aseptischen vorbereitung (t1) und nach der Operation (t2) entnommen. Die Gewebsreaktion wurde zum

Zeitpunkt t1 überwacht. Die Proben wurden im Hinblick auf kultivierbare aerobe und anaerobe Bakterien und Pilze analysiert. Die Prävalenz von positiven Kulturabstrichen wurde verwendet, um die Wirksamkeit von PI zu messen. In der MRSA Studie wurden die Bindehautabstriche gesammelt, unmittelbar bevor ophthalmochirurgische Eingriffe erfolgten, und die Identifizierung und Empfindlichkeitsprüfung von *S. aureus* verdächtigen Kolonien, wurde mit Vitek2 (bioMérieux, Lyon) durchgeführt und das Vorhandensein von MRSA durch PCR verifiziert. Die genetische Verwandtschaft aller MRSA-Isolate wurde mittels Pulsfeld-Gelelektrophorese (PFGE) und Spa-Typisierung untersucht.

Das Ergebnis der PI Studie an Kleintiere zeigte, dass Lösungen mit 0,2% und 1% PI verwendet werden könnten, um eine bakterielle Kontamination von Augenoberflächengewebe ohne Gewebereaktion zu eliminieren. 0,2% PI sind hierbei wirksamer als konzentriertere Lösungen (p = 0,02). Zusätzlich reduzierte eine Konzentration von 0,2% PI *Bacillus sp*. und Koagulase-negative *Staphylococcus sp*. Zu den Zeitpunkten t1 und t2. In diesen Teil der Studie würde kein MRSA nachgewiesen. Aus diesem Grund wird die Wirksamkeit von PI zur Eliminierung von MRSA auf der Augenoberfläche von Tieren nicht beurteilt werden.

Die Ergebnisse der MRSA Studie zeigten, dass die Isolierungsraten mit 6/46 Proben hoch waren (13%). Die molekulare Typisierung ergab, dass alle detektierten MRSA vom Typ t011 sind. Dieser Typ wird üblicherweise mit der europäischen, mit Tieren assoziierten MRSA-Linie des klonalen Komplexes CC 398 in Verbindung gebracht, die kürzlich auch bei Pferden in Deutschland aufgetreten ist. Darüber hinaus konnte mit der PFGE nachgewiesen werden, dass die sechs MRSA-Keime sehr nah miteinander verwandt waren (> 90%). Nach der Anamnese der Patienten und dem Ergebnis der mikrobiologischen Untersuchung, lässt sich vermuten, dass dieses Phänomen durch eine nosokomiale Infektion begründet war. Die in unserer Studie kultivierten MRSA zeigten weitere Resistenzen gegenüber Antibiotika, die

häufig zur topischen Augenbehandlung verwendet werden, wie z.B. Fluorchinolone, Tetracycline, Kanamycin und Gentamicin.

Die effektivste Konzentration von PI wurde mit 0,2% nachgewiesen. Es zeigte eine ausgezeichnete antibakterielle Aktivität gegen *Bacillus* sp. und Koagulase-negative *Staphylococcus sp*. beim Kleintier. Diese Studie zeigte eine hohe Prävalenz von MRSA auf den Augenoberflächen von equinen Patienten, die die Notwendigkeit einer konstanten bakteriellen Überwachung unterstützen.

Weitere Studien zur Quelle von MRSA bei equinen ophthalmologischen Patienten, einschließlich Umweltaspekten, standardisierten Vorgehenssverfahren (SOPs) und Manipulationen sowie diagnostische Ansätze sind erforderlich. Es ist notwendig, die bakterizide Aktivität von Antiseptika gegen MRSA auf der Augenoberfläche des Tieres entweder mit höheren Konzentrationen von PI oder alternativen Protokollen und aktiven Komponenten zur Verringerung der Kontamination der Augenoberfläche zu beurteilen.

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II List of abbreviations

CC	**C**lonal **C**omplex
CFU	**C**olony **F**orming **U**nit
CLSI	**C**linical and **L**aboratory **S**tandard **I**nstitute
Con	**C**oncentration
DC	**D**og in the **C**ommunity
DCC	**D**og and **C**at in the **C**ommunity
DCVA	**D**og and **C**at in **V**eterinary hospital **A**dmission
DVA	**D**og in **V**eterinary hospital **A**dmissions
ENR	**Enr**ofloxacin
ESBL	**E**xtended **S**pectrum **B**eta-**L**actamase producing *Escherichia coli*
et al.	**et al**ii (latin for “and others”)
GEN	**Gen**tamicin
Group	equine patient **group**
HA	**H**orse in hospital **A**dmissions
HEH	**H**orse stationary in **E**quine **H**ospital
HC	**H**orse in the **C**ommunity
HF	**H**orse in **F**arm
ID	**I**dentification **N**umber
IEC	**I**mmune **E**version **C**luster

J	**Y**ear
KAN	**Kan**amycin
L	**L**eft
M	**M**onth
MAR	**Mar**bofloxacin
MCG	**M**aximum **C**ommon **G**enome
MDR	**M**ulti **D**rug **R**esistant
MIC	**M**inimal **I**nhibitory **C**oncentrations
MLST	**M**ulti-**L**ocus **S**equence **T**ype
MRSA	**M**ethicillin-**R**esistant ***S****taphylococcus* ***A****ureus*
MRSP	**M**ethicillin-**R**esistant ***S****taphylococcus* ***P****seudintermedius*
NaCl	sodium chloride
n.a	**n**ot **a**pplicable
p	***p*** value
PBP	**P**enicillin **B**inding **P**rotein
PCR	**P**olymerase **C**hain **R**eaction
PFGE	**P**ulsed – **F**ield **G**el **E**lectrophoresis
PI	**P**ovidone-**I**odine
R	**R**ight
S*. aureus*	***S****taphylococcus aureus*

SCIN	**s**taphylococcal **c**omplement **i**nhibitor
SOPs	**S**tandard **O**peration **P**rocedures
Spa	protein A encoding gene *spa*-type
ST	**S**equence **T**ype
t0	timepoint before aseptic preparation
t1	timepoint after aseptic preparation
t2	timepoint after surgery
TET	**Tet**racycline
vWbp	**W**illebrand-factor **b**inding **p**rotein
WGS	**W**hole **G**enome **S**equencing

III Appendices

Appendix A

Table 1: Recommendations for aseptic technique in veterinary ophthalmology surgery

Author	**Type of literat.**	**Around the surgical field (Skin)**			**Eyelids**		**Eye lid margin**				**Ocular surface (Conjunctiva, Cornea)**	
		Betadine Surgical Scrub	NaCl	PI	Baby Shampoo	PI	PI	Chlorhexidine gluconate 0.05%	Isopropyl alcohol 4%	Cutasept®	Poly-hexanid 0.02% (PX)	NaCl
Bistner, S.I. 1977	VOS	7.5%	✓				1.0%					✓ and 1:2 PI
Blogg, J.R. 1980	VO			✓		✓						✓ or dilute betadine
Slatter, D.H. 2001	VO			1:25								✓ and 1:25-1:50 PI
Martin, C.L. 2005	VO			1.0%			1.0%					✓
Gelatt, K.N. 2011	VOS		✓					✓	✓			✓ and 0.5% PI
Tobias, K.M. 2012	SAS			0.2%			1:50					✓ and PI

Table 1: Recommendations for aseptic technique in veterinary ophthalmology surgery (Con't)

Author	**Type of literat.**	**Around the surgical field (Skin)**			**Eyelids**		**Eye lid margin**				**Ocular surface (Conjunctiva, Cornea)**	
		Betadine Surgical Scrub	NaCl	PI	Baby Shampoo	PI	PI	Chlorhexidine gluconate 0.05%	Isopropyl alcohol 4%	Cutasept®	Poly-hexanid 0.02% (PX)	NaCl
Fossum, T.W. 2013	SAS			✓			✓					✓
Gelatt, K.N. 2014	VO		✓		1:20		1:50					✓
Gould, D. 2014	VO			1:10								✓ and 1:50 PI
Robert, S.M. 1986	SP		✓	0.2%		0.2%	0.2%					✓
Hansmann, F. 2004	SP									✓	✓	✓ and 1.25%

SAS: Small Animal Surgery, VOS: Veterinary Ophthalmic Surgery, VO: Veterinary Ophthalmology, SP: surgical paper, PI: Povidone Iodine, NaCl: 0.9% Saline Solution, ✓ = recommended without specification, Cutasept® = 2-Propanol and Benzalkonium)

Appendix B:

Table 2: The general signalment of small animals undergoing enucleation in pilot study.

Animal ID	Species*	Date	Sex+	Age++	Weight (kg.)	Breed**	Eye disease#	Sample site##	Concentration (%) of PI
1A	1	26.01.2016	Mc	2Y9M	9.0	8	8	R	**0.1**
2A	1	18.03.2016	Fc	17Y9M	6.9	1	9	L	**0.1**
3A	1	15.03.2016	M	7Y5M	6.5	9	9	R	**0.1**
4A	2	17.03.2016	Mc	16Y8M	5.2	6	10	R	**0.1**
5A	1	17.03.2016	Mc	12Y8M	18.0	10	9	R	**0.1**
6A	1	24.05.2016	Fc	12Y	8	1	11	L	**0.1**
7A	2	19.07.2016	Fc	8Y4M	4.3	6	2, 12	R	**0.1**
8A	2	26.07.2016	F	6Y8M	4.2	6	9	R	**0.1**
9A	2	09.09.2016	F	1Y4M	3.2	6	8	R	**0.1**
10A	2	27.09.2016	Fc	9Y10M	12.0	11	12	R	**0.1**
1B	1	29.09.2015	Fc	6Y3M	12.6	1	1,2	R	**0.2**
2B	1	13.10.2015	Fc	6Y10M	11.5	1	3,2	L	**0.2**
3B	1	17.11.2015	Fc	6Y5M	26.0	1	4	R	**0.2**
4B	1	08.12.2015	Fc	5Y5M	7.5	2	5	R	**0.2**
5B	1	22.12.2015	F	3Y8M	9.0	2	5	L	**0.2**
6B	1	07.06.2016	F	5Y1M	4.4	3	2, 6	L	**0.2**
7B	1	26.07.2016	Mc	5Y2M	7.5	4	6	L	**0.2**
8B	1	06.09.2016	F	14Y0M	7.2	5	2,6	R	**0.2**
9B	2	23.09.2016	F	0Y5M	2.2	6	7	R	**0.2**
10B	1	18.10.2016	Mc	16Y10M	8.5	7	8	R	**0.2**
1C	1	22.03.2016	M	4Y3M	37.0	1	1	R	**1**
2C	1	23.03.2016	F	7Y8M	11.8	11	7	L	**1**
3C	2	31.03.2016	Fc	6Y4M	4.27	6	2, 12, 13	L	**1**
4C	1	26.04.2016	Mc	4Y8M	21.0	1	14, 15	R	**1**
5C	2	26.04.2016	Mc	13Y8M	3.5	6	16,17	L	**1**
6C	2	07.06.2016	F	N/A	3	12	8	R	**1**
7C	2	23.09.2016	F	N/A	4	6	8	L	**1**
8C	2	28.09.2016	F	N/A	N/A	6	19	R	**1**

Animal ID	Species*	Date	Sex+	Age++	Weight (kg.)	Breed**	Eye disease#	Sample site##	Concentration (%) of PI
9C	2	04.10.2016	Fc	7Y9M	17.0	13	1	L	**1**
10C	2	17.10.2016	M	10J6M	5.8	6	2, 20	R	**1**
1D	2	28.04.2016	Mc	13Y3M	5.9	14	9	L	**5**
2D	2	03.05.2016	Mc	6Y0M	4.5	6	21	L	**5**
3D	1	03.05.2016	Mc	8Y3M	16.0	1	9	R	**5**
4D	1	04.05.2016	F	7M	2.2	8	8	L	**5**
5D	1	18.05.2016	Fc	9Y5M	13.0	11	9	R	**5**
6D	1	22.11.2016	Fc	6Y10M	2.2	15	8	L	**5**
7D	2	22.11.2016	M	14Y10M	5.4	6	6, 20	R	**5**
8D	1	22.11.2016	M	4Y5M	13.5	1	2, 20	L	**5**
9D	1	30.11.2016	M	10Y10M	7	1	8	R	**5**
10D	1	29.11.2016	M	8Y1M	14	11	9	L	**5**

List of Abbreviations:

n/a = not applicable

*Specie: 1 = Dog, 2 = Cat

+Sex: F= Female, Fc = castrated Female M= Male, Mc= castrated male

++Age: Y = year, M = Month

**Breed: 1 = Mixed, 2 = Pug, 3 = Jack Russel terrier, 4 = Patterdale Terrier, 5 = Dachsbracke, 6 = European short hair, 7 = Bolognese, 8 = Shih Tzu, 9 = Italian grays how, 10 = Beagle, 11 = French Bulldog, 12 = Bengal, 13 = Magyar Vizsla, 14 = British short hair, 15 = Yorkshire Terrier

#Eye disease: 1 = Traumatic uveitis, 2 = Secondary glaucoma, 3 = Hyper mature cataract, 4 = Primary glaucoma, 5 = Corneal ulcer, 6 = Anterior lens luxation, 7 = Corneal perforation, 8 = Bulbus prolapse, 9 = Glaucoma, 10 = retrobulbar process, 11 = Secondary uveitis, 12 Uveitis, 12 = Chronic corneal erosion, 13 = Septic implantation, 14 = Cataract, 15 = Retinal detachment, 16 = Keratitis, 17 = Chronic glaucoma, 19 = Bulbus trauma, 20 = Chronic Uveitis, 21 = Exposition keratitis

##Sample site, R = Right, L = Left

Appendix C:

Table 3: The microbiological result on ocular surfaces of small animals undergoing enucleation in pilot study

Animal ID	Sample site*	Microbiota t1**(Germ content***)	Microbiota t2**(Germ content***)	Microbiota t3**(Germ content***)	Tissue Reaction
1A	R	2(++), 18(++), 19(++)	12(+), 18(++)	7	no
2A	L	29(++)	7	7	no
3A	R	negative	negative	negative	no
4A	R	21(++), 20(++)	negative	negative	no
5A	R	negative	12(+)	negative	no
6A	L	12(+), 22(++)	12(+)	3(+), 12(+)	no
7A	R	23(+++++)	23(+++)	23(+++)	no
8A	R	24(+), 6(+)	negative	negative	no
9A	R	2(+++++), 25(+++++), 26(+++++)	2(+++++), 25(+++++), 26(++++)	12(+)	no
10A	R	2(+++), 21(+++), 27(+)	2(+)	2(+++)	no
1B	R	1(++), 2(++)	3(+)	negative	no
2B	L	2(++), 4(+++), 5(+++)	negative	negative	no
3B	R	6(++)	7	7	no
4B	R	8(++), 9(+)	negative	negative	no
5B	L	2(+++), 10(+++), 11(++)	2(++)	2(+++)	no
6B	L	3(++), 12(+), 13(+)	3(+)	6(++)	no
7B	L	2(++), 12(+)	negative	negative	no
8B	R	13(+++), 14(++++), 15(++)	negative	negative	no
9B	R	7	negative	negative	no
10B	R	16(++), 17(+)	negative	negative	no
1C	R	2(+), 28(++), 12(+)	negative	negative	no
2C	L	19(###), 25(+++++)	25(++)	2(++), 25(++)	no
3C	L	7	7	7	no
4C	R	12(++), 19(++), 30(+)	negative	12(++)	no
5C	L	3(+), 31(++)	negative	negative	no
6C	R	28(+++++), 32(+++), 33(++++), 35(+++)	18(++), 32(++), 33(++)	35(+)	no
7C	L	12(++++), 24(###), 30(###), 36(++++), 37(++++)	12(+++), 24(+++), 30(###), 36(++++), 37(++++)	12(++), 24(+++), 30(+++), 36(+++), 37(++++)	no
8C	R	12(++++), 23(+++++), 36(++++)	negative	negative	no
9C	L	2(++), 38(++)	negative	negative	no
10C	R	12(+), 39(+)	negative	negative	no

Animal ID	Sample site*	Microbiota t1**(Germ content***)	Microbiota t2**(Germ content***)	Microbiota t3**(Germ content***)	Tissue Reaction
1D	L	36(+++++)	36(+++++)	36(+++++)	yes
2D	L	2(+++), 13(++), 19(+++)	negative	40(++), 12(++), 19(++), 40(++)	yes
3D	R	2(+++), 12(+)	negative	2(+), 12(+)	yes
4D	L	2(++), 41(+++)	negative	negative	yes
5D	R	2(+++++)	42(+)	2(++), 12(+)	yes
6D	L	3(+), 12(++)	negative	negative	yes
7D	R	21(+)	negative	negative	yes
8D	L	2(+++++), 12(++)	2(++)	2(+++), 21(++)	yes
9D	R	12(+), 15(++), 24(++)	negative	negative	yes
10D	L	7	negative	negative	yes

List of Abbreviations:

*Sample site: R = Right, L = Left

**Microbiota: 1 = *Staphylococcus warneri*, 2 = *Staphylococcus sp. Intermedius* group, 3 = *Staphylococcus hominis*, 4 = *Enterobacter kobi*, 5 = *Clostridium perfrigens*, 6 = *Staphylococcus epidermidis*, 7 = Isolated unspecific species of bacteria, 8 = *Pseudomonas aeruginosa*, 9 = *Enterococcus hirae*, 10 = *Yeast* sp., 11 = *Alternaria* sp., 12 = *Bacillus* sp., 13 = *Enterococcus farcium*, 14 = *Streptococcus minor*, 15 = *Actinomyces* sp, 16 = *Moraxella canis*, 17 =*Microbacteriam* sp, 18 = *Staphylococcus* sp. Saprophyticus group, 19 = *Staphylococcus haemolyticus*, 20 = *Staphylococcus capitis*, 21 = *Corynebacterium* sp., 22 = *Bergeyella* sp., 23 = *Staphylococcus felis*, 24 = *Staphylococcus xylosus*, 25 = *Staphylococcus aureus*, 26 = *Streptococcus dysgalactiae*, 27 = *Staphylococcus capitis*, 28 = *Enterococcus faecalis*, 29 = *Clostridium sordellii*, 30 = *Streptococcus canis*, 31 = *Micrococcus* sp., 32 = *Enterobacter* cloacce, 33 = *Staphylococcus sciuri*, 34 = *Actinobacter haemolyticus*, 35 = *Actinobacter* sp., 36 = *Pasteurella multocida*, 37 = *Prevotella* sp., 38 = *Staphylococcus caprae*, 39 = *Rhodococcus* sp., 40 = *Escherichia coli*, 41 = *Enterobacter aerogenes*, 42 = *Paenibacillus odorifer*

***Germ content: + = over enrichment broth, ++ = 1-2 colony, Isolated, +++ = low content, ### = low – moderate content, ++++ = moderate content, #### = moderate – high content, +++++ = high content

Appendix D:

Table 4: The general signalment of small animals undergoing ophthalmic manipulation

Animal ID	Species*	Date	Sex+	Age++	Weight (kg.)	Breed**	Eye disease#	Sample site##	Manipulation###
1	1	13.10.2015	F	5Y8M	6.5	1	1	R	1
1	1	13.10.2015	F	5Y8M	6.5	1	1, 2	L	1
2	1	13.10.2015	Fc	8Y3M	15.5	2	1	L	1
3	1	27.10.2015	F	8Y4M	28.5	3	3	L	2
4	1	01.12.2016	M	4Y8M	14.0	2	4,5	R	3
5	1	08.12.2015	F	4Y8M	2.7	4	6	L	4
6	1	29.12.2015	F	1Y10M	9.3	5	6	R	4
7	1	29.12.2015	Mc	7Y11M	13.5	2	3	R	2
8	2	05.01.2016	M	11Y11M	3.9	6	7	L	5
9	1	12.01.2016	M	9Y8M	6.3	7	8	L	6
10	1	12.01.2016	Mc	3Y3M	23.0	2	9	R	7
10	1	12.01.2016	Mc	3Y3M	23.0	2	9	L	7
11	1	12.01.2016	F	1Y4M	27.5	8	10	L	8
12	1	19.01.2016	F	7Y2M	8.1	5	4, 5, 11	R	7, 9
12	1	19.01.2016	F	7Y2M	8.1	5	4, 5, 11	L	7, 9
13	1	19.01.2016	M	N/A	11.1	5	12	R	10
14	2	26.01.2016	F	6Y8M	5.5	9	13	R	8
15	1	26.01.2016	Mc	2Y9M	9.0	10	4, 12	L	7, 10
16	1	26.01.2016	Mc	7Y4M	31.0	11	3	2	11, 2
16	1	26.01.2016	Mc	7Y4M	31.0	11	1	L	1
17	1	02.02.2016	F	5Y7M	11.9	5	4, 5	R	12
17	1	02.02.2016	F	5Y7M	11.9	5	1, 2, 4, 5	L	12
18	2	09.02.2016	Mc	1Y4M	4.2	12	9, 14	R	2,7
18	2	09.02.2016	Mc	1Y4M	4.2	12	9, 14	L	2, 7
19	1	09.02.2016	F	3Y5M	19.2	13	1, 9	R	7
19	1	09.02.2016	F	3Y5M	19.2	13	1, 9	L	7
20	1	12.02.2016	M	8Y1M	3.5	4	15	R	13
21	1	22.02.2016	M	10Y4M	9.8	14	16	R	8

Animal ID	Species*	Date	Sex+	Age++	Weight (kg.)	Breed**	Eye disease#	Sample site##	Manipulation###
21	1	22.02.2016	M	10Y4M	9.8	14	16	L	8
22	1	01.03.2016	M	4Y11M	10.1	5	4, 5, 11	R	9, 12
22	1	01.03.2016	M	4Y11M	10.1	5	4, 5, 11	L	9, 12
23	1	01.03.2016	Mc	11Y8M	11.7	15	12	L	10
24	1	08.03.2016	F	9M	15.0	16	9	R	14
25	1	08.03.2016	Fc	2Y3M	9.2	17	17	L	15
26	1	15.03.2016	Fc	2Y1M	3.5	18	1	R	1, 9
26	1	15.03.2016	Fc	2Y1M	3.5	18	1, 18	L	1, 9
27	1	14.03.2016	M	10Y2M	8.8	19	12	R	10
28	1	15.03.2016	M	1Y0M	21.0	16	9	R	16
28	1	15.03.2016	M	1Y10M	21.0	16	9	L	16
29	2	21.03.2016	Fc	6Y4M	4.27	12	19	L	17
30	1	05.04.2016	F	8M	15.5	5	17	R	15
30	1	05.04.2016	F	8M	15.5	5	17	L	15
31	1	12.04.2016	M	8Y1M	3.5	4	15	R	18
32	1	19.04.2016	M	11Y8M	22	17	12	L	10
33	1	19.04.2016	F	3Y7M	11	20	16	R	8
34	1	26.04.2016	Mc	13Y4M	9	1	1, 13	L	8
35	1	03.05.2016	F	9Y1M	7	14	16	R	8
35	1	03.05.2016	F	9Y1M	7	14	16	L	8
36	1	17.05.2016	M	1Y2M	10	17	4, 5, 20	R	19
37	1	17.05.2016	Fc	2Y3M	9.2	17	17	L	15
38	2	23.05.2016	Mc	8Y8M	6.5	21	2, 7	L	5
39	1	24.05.2016	F	4Y1M	7	22	17	L	15
40	2	01.06.2016	Mk	14Y8M	6.8	12	22	L	20
41	1	31.05.2016	M	6Y8M	6.5	10	13	R	8
42	1	01.05.2016	Fc	9Y0M	12	20	21	L	13
43	1	01.06.2016	M	4Y3M	13.5	17	23, 24	L	8
44	1	07.06.2016	M	4Y3M	13.5	17	25	L	17
45	1	07.06.2016	Fc	11Y8M	10	23	126	R	10
46	1	07.06.2016	M	7Y11M	50	24	2, 9	R	7
47	1	10.06.2016	F	4Y1M	4.5	25	27	L	21
48	1	14.06.2016	Fc	10Y8M	37	26	12	R	10
49	1	14.06.2016	F	2Y10M	21	2	1, 4	R	1
49	1	14.06.2016	F	2Y10M	21	2	1, 4	L	1
50	2	14.06.2016	Fc	9Y8M	5.8	21	7	R	5
51	2	21.06.2016	Fc	8Y8M	4.6	21	7	R	5

Animal ID	Species*	Date	Sex+	Age++	Weight (kg.)	Breed**	Eye disease#	Sample site##	Manipulation###
51	2	21.06.2016	Fc	8Y8M	4.6	21	7	L	4, 5
52	1	27.06.2016	Fc	2Y6M	6.35	27	13	R	8
52	1	27.06.2016	Fc	2Y6M	6.35	27	13	L	8
53	1	13.07.2016	F	0Y8M	15.5	5	17	L	15
54	1	13.07.2016	Fc	11Y6M	7.5	28	16, 22	R	8
54	1	13.07.2016	Fc	11Y6M	7.5	28	16, 22	L	8
55	1	19.07.2016	F	1Y4M	21.5	29	1, 9	R	1, 7
55	1	19.07.2016	F	1Y4M	21.5	29	1, 9	L	1, 9
56	1	26.07.2016	Mc	5Y2M	7.5	30	23	R	21

List of Abbreviations:

n/a = not applicable

*Specie: 1 = Dog, 2 = Cat

+Sex: F= Female, Fc = castrated Female M= Male, Mc= castrated male

++Age: Y = year, M = Month

**Breed: 1 = Mini Poodle, 2 = French bulldog, 3=Boxer, 4 = Chihuahua, 5 = Pug, 6 = Persia, 7 = Maltese, 8 = Golden retriever, 9 = Main coon, 10 = Shi-Tsu, 11 = English bulldog, 12 = European short hair, 13 = Shar Pei, 14 = Long haired teckel, 15 = Beagle, 16 = Labrador, 17 = Mixed Breed, 18 = Toy poodle, 19 = West highland white terrier, 20 = Cocker spaniel, 21 = British shorthair, 22 = Schnauzer, 23 = Short hair teckel, 24 = Greater Swiss Mountain, 25 = Jack Russel Terrier, 26 = Rottweiler, 27 = Peking, 28 = Boston Terrier, 29 = American Staffordshire, 30 = Patterdale Terrier

#Eye disease: 1 = Distichiasis, 2 = Corneal erosion, 3 Chronic corneal erosion, 4 = Macro blepharon, 5 = Nasal entropion, 6 = Corneal perforation, 7 = Corneal sequester, 8 = Keratoconjunctivitis sicca, 9 = Entropion, 10 = Juvenile Cataract, 11 = Keratitis Pigmentosa, 12 = Eyelid tumor, 13 = Mature cataract, 14 = Microphthalmia, 15 = Facial nerve paralysis, 16 = Immature Cataract, 17 = Cherry eye, 18 = Nasolacrimal duct stenosis, 19 = Uveitis, 20 = Ectopic cilia, 21 = Retrobulbar process, 22 = Squamous cell carcinoma, 23 = Cataract, 24 = Lens induce uveitis, 25 = Chronic uveitis, 26 = Third eyelid tumor, 27 = Anterior lens luxation, 22 = Iris cyst, 23 = Posterior lens luxation

##Sample site: R = Right, L = Left

###Manipulation: 1= Distichiasis excision, 2 = Third eyelid flap, 3 = Medial canthus technique, 4 = Conjunctival flap, 5 = Keratectomy, 6 = Tran positioned ductus parotid to conjunctival sac, 7 = Eye lid correction, 8 = Extra capsular lens extraction, 9 = Cryosurgery, 10 = Eyelid tumor excision, 11 = Corneal debridement, 12 = Nasal Canthus Plastic, 13 = Tarsorrhaphy, 14 = Tacking technique, 15 = Pocket technique, 16 = Injected hyaluronic acid in lower eyelid, 17 = Injected TPA in anterior chamber, 18 = Performed smaller eyelid open, 19 = Ectopic cilia excision, 20 = Lip to lid plastic, 21 = Intra capsular lens extraction

Appendix E:

Table 5: The microbiological result on ocular surfaces of small animals undergoing ophthalmic manipulation

Animal ID	Sample site*	Microbiota t1**(Germ content***)	Microbiota t2**(Germ content***)	Microbiota t3**(Germ content***)
1	R	43(+++++)	43(###)	43(###)
	L	negative	negative	negative
2	L	2(+++++)	2(++++)	2(###)
3	L	1(+++), 6(+++)	negative	12(++)
4	R	2(###), 30(+++)	2(+++), 30(+++)	30(+)
5	L	6(+)	negative	negative
6	R	2(+++)	2(####)	2(+++)
7	R	7	negative	negative
8	L	23(++)	7	negative
9	L	30(+++), 43(++)	30(####), 43(++)	30(####), 43(+++)
10	R	2(++), 16(++), 30(###), 40(+++)	30(++++), 40(####)	2(++), 30(++)
	L	30(+++), 40(++++)	2(++), 30(+++), 40(+++), 43(++)	30(+++),40(+++)
11	L	negative	negative	negative
12	R	2(++++), 30(+++), 44(###)	2(+++), 44(++)	2(+++), 16(###)
	L	2(###), 30(+++), 45(+++)	2(++), 44(+)	2(+++)
13	R	2(++++), 30(+++)	2(+), 30(+)	2(++)
14	R	46(++)	negative	6(++)
15	L	47(++)	negative	1(++), 6(++)
16	2	12(+), 30(+), 47(+)	negative	negative
	L	negative	1(+)	6(+)
17	R	1(+++), 2(###), 30(++), 48(####)	2(++)	2(++), 30(+)
	L	2(++), 3(++), 30(++), 48(++++)	2(+++), 30(###)	2(++)
18	R	23(++++), 30(++++), 36(++++)	23(++), 30(++), 36(++)	23(++), 30(++), 36(++)
	L	23(++), 36(+)	21(++), 30(++), 36(++)	23(+++), 30(++++), 36(++++)
19	R	2(++),19(++)	negative	negative
	L	19(+++), 49(++)	2(++)	18(++)
20	R	6(++), 44(+)	negative	6(+)
21	R	2(++), 10(++)	negative	negative
	L	1(++), 32(+)	32(++)	32(++)
22	R	2(+++++), 12(++), 21(++), 30(+++++)	2(++), 21(++)	12(++), 21(++)

Animal ID	Species*	Date	Sex+	Age++
	L	2(++++), 21(++++), 30(++++)	2(+++++), 21(++), 30(++)	16(++), 21(++)
23	L	2(+++), 18(+++), 30(++)	negative	18(+)
24	R	2(+)	negative	12(++)
25	L	50(++), 51(++), 52(++)	negative	negative
26	R	7	53(++)	7
	L	3(+), 12(+), 49(+)	12(+)	negative
27	R	2(++++)	2(+)	2(+)
28	R	12(++), 30(+++++)	30(+++)	12(++), 30(++)
	L	12(++++), 30(####)	30(+++)	30(+)
29	L	1(+)	negative	negative
30	R	2(++), 49(+++++)	7	7
	L	2(++++), 49(###)	negative	12(++)
31	R	4(++), 51(####)	51(++), 54(++)	54(+)
32	L	1(++), 2(+++)	1(++)	1(++), 6(++)
33	R	2(++)	2(++)	2(++)
34	L	2(++), 12(++)	2(++), 12(++)	negative
35	R	6(++)	6(++)	2(++)
	L	7	negative	negative
36	R	19(++)	negative	negative
37	L	2(+++++), 13(++)	negative	negative
38	L	3(+)	negative	negative
39	L	2(+++++), 55(+++)	55(+++)	2(+), 55(###)
40	L	negative	negative	negative
41	R	6(++), 12(+)	negative	negative
42	L	12(+)	negative	negative
43	L	2(+)	negative	negative
44	L	negative	negative	negative
45	R	2(+++++)	2(###)	2(###)
46	R	22(++), 49(++), 56(+)	negative	negative
47	L	2(++), 19(++), 21(####)	2(+)	negative
48	R	12(+)	negative	negative
49	R	2(###), 8(+++)	2(+)	2(+)
	L	2(++++),12(++)	2(++)	2(++)
50	R	negative	negative	negative
51	R	23(++++)	23(+++)	23(+++)
	L	23(+++++)	negative	23(++)
52	R	negative	negative	negative

Animal ID	Species*	Date	Sex+	Age++
	L	3(++)	negative	negative
53	L	51(++)	12(+)	12(++)
54	R	negative	negative	negative
	L	3(+++), 57 = (+++)	negative	negative
55	R	12(+), 19(+)	negative	negative
	L	12(+), 13(+), 51(+)	negative	negative
56	R	12(++), 56(++), 58(++)	negative	negative

List of Abbreviations:

*Sample site: R = Right, L = Left

**Microbiota: 1 = *Staphylococcus warneri*, 2 = *Staphylococcus sp. Intermedius* group, 3 = *Staphylococcus hominis*, 4 = *Enterobacter kobi*, 5 = *Clostridium perfrigens*, 6 = *Staphylococcus epidermidis*, 7 = Isolated unspecific species of bacteria, 8 = *Pseudomonas aeruginosa*, 9 = *Enterococcus hirae*, 10 = *Yeast* sp., 11 = *Alternaria* sp., 12 = *Bacillus* sp., 13 = *Enterococcus farcium*, 14 = *Streptococcus minor*, 15 = *Actinomyces* sp., 16 = *Moraxella canis*, 17 =*Microbacteriam* sp., 18 = *Staphylococcus* sp. Saprophyticus group, 19 = *Staphylococcus haemolyticus*, 20 = *Staphylococcus capitis*, 21 = *Corynebacterium* sp., 22 = *Bergeyella* sp., 23 = *Staphylococcus felis*, 24 = *Staphylococcus xylosus*, 25 = *Staphylococcus aureus*, 26 = *Streptococcus dysgalactiae*, 27 = *Staphylococcus capitis*, 28 = *Enterococcus faecalis*, 29 = *Clostridium sordellii*, 30 = *Streptococcus canis*, 31 = *Micrococcus* sp., 32 = *Enterobacter* cloacce, 33 = *Staphylococcus sciuri*, 34 = *Actinobacter haemolyticus*, 35 = *Actinobacter* sp., 36 = *Pasteurella multocida*, 37 = *Prevotella* sp., 38 = *Staphylococcus caprae*, 39 = *Rhodococcus* sp., 40 = *Escherichia coli*, 41 = *Enterobacter aerogenes*, 42 = *Paenibacillus odorifer*, 43 = *Serratia marcescens*, 44 = *Pasteurella* sp., 45 = *Pasteurella dagmatis*, 46 = *Staphylococcus pettenkoferi*, 47 = *Staphylococcus* spp., 48 = *Malassezia* sp., 49 = *Staphylococcus equorum*, 50 = *Streptococcus mitis*, 51 = *Rothia* sp., 52 = *Pasteurellaceae* sp. 53 = *Penicillium* sp., 54 = *Candida albicans*, 55 = *Klebsiella pneumoniae*, 56 = *Neisseria* sp., 57 = *Trichosporon* sp., 58 = *Arthrobacter* sp

***Germ content: + = over enrichment broth, ++ = 1-2 colony, Isolated, +++ = low content, ### = low – moderate content, ++++ = moderate content, #### = moderate – high

Appendix F

Table 6: The general signalment and microbiological result on ocular surfaces of horses undergoing ophthalmic manipulation.

Horse ID	Date	Sex+	Age++	Weight (kg.)	Breed*	Eye disease**	Sample Site***	Manipulation#	Microbiota## (Germ content###)
1	29.10.2015	F	5Y4M	610	1	1	L	1	1(++), 2(+)
2	15.10.2015	Mc	4Y4M	570	2	2	R	2	3
3	15.10.2015	F	4Y5M	n/a	3	3	R	3	4(+++), 5(++), 22(++)
4	30.11.2015	F	5Y5M	575	4	4	R	4	3, 6(++)
5	09.10.2015	F	10Y4M	n/a	5	4	L	4	4(+++), 7(++++), 8(++), 23(+++)
6	03.12.2015	Mc	8Y6M	750	6	4	L	4	4(++), 9(+++), 10(+++),24(+++), 25(+++)
7	07.12.2015	Mc	8Y6M	n/a	7	4	R	4	1(++++ - +++++), 2(+++), 5(++++), 6(+++), 26(+++++)
8	08.12.2015	F	11Y5M	600	3	4	R	4	11(+++-++++), 22(+++)
8	08.12.2015	F	11Y5M	600	3	4	L	4	4(++)
9	10.12.2015	F	19Y5M	450	8	4	L	4	4(+++), 27(+++)
10	15.12.2016	Mc	22Y6M	620	9	1	L	6	4(+), 28(++)
11	17.12.2015	F	14Y4M	n/a	10	4	L	4	4(++), 12(++)
12	05.01.2016	F	18Y8M	600	9	5	R	7	3
10	21.01.2016	Mc	22Y6M	620	9	1	L	8	4(++), 10(++), 13(+++++), 14(+), 15(++)
13	28.01.2016	M	5Y4M	n/a	11	4	L	4	27(++), 4(++), 7(++)
14	29.01.2016	F	12Y5M	615	3	6	R	9	14(++), 27(+++)
15	01.02.2016	F	9Y5M	330	11	1	R	1	4(++), 26(+++)
16	04.02.2016	Mc	20Y8M	500	12	7	R	2	4(++), 14(++)
17	15.02.2016	F	24Y5M	400	13	3	L	3,8,10	14(++), 15(++), 16(++), 24(++)
18	01.03.2016	F	4Y5M	450	14	2	R	8	4(+), 17(+)
19	14.03.2016	Mc	10Y6M	590	1	4	L	4	4(++), 23(++)
20	10.03.2016	Mc	7Y4M	335	11	4	L	4	4(++), 26(+++)
21	24.03.2016	F	24Y4M	n/a	15	4	L	4	4(++), 15(++++), 25(++), 29(++)
22	07.04.2016	Mc	13Y6M	620	16	8	L	4	13(+++++), 18 (+++), 19 (++++-+++++), 20(++), 23(+++)
23	12.04.2016	F	9Y4M	370	11	4	R	4	4(+++), 15(+++), 29(+++)
24	21.04.2016	M	12Y2M	315	11	9	R	11	21(+), 22(++), 24(+++), 29(+++)
25	21.04.2016	F	16Y8M	490	17	4	R	4	1(+++), 4(+++), 15(+++)
26	28.04.2016	Mc	19Y6M	600	18	4	L	4	4(++), 25(++), 26(++)
25	17.05.2016	F	16Y8M	490	17	1	R	12	1(+++++), 22(++)
25	17.05.2016	F	16Y8M	490	17	4	L	12	4(+), 8(++), 15(+), 30(++)

Horse ID	Date	Sex+	Age++	Weight (kg.)	Breed*	Eye disease**	Sample Site***	Manipulation#	Microbiota## (Germ content###)
27	25.05.2016	Mc	16Y8M	405	19	8	R	5	4(++), 18(+++), 25(+)
28	02.06.2016	Mc	9Y4M	n/a	10	4	R	4	4(+++), 10(+++),23(++), 27(++)
29	05.07.2016	M	4Y8M	400	11	10	L	5	2(++), 8(+), 31(+), 32(++)
30	30.06.2016	F	23Y4M	n/a	20	4	R	4	4(+), 21(+++), 23(+++), 25(+++)
31	26.07.2016	Mc	15Y5M	730	2	10	R	7	5(++), 11(++), 23(+++), 26(+++), 33(+++), 34(+++), 35(+++++)
32	03.12.2015	F	18Y5M	520	9	1, 11	L	13	4(+++++), 7(+++), 8(++), 10(+++), 26(+++++)
33	18.12.2015	F	28Y8M	255	19	1, 11	R	13	4(++), 10(++), 36(++)
34	22.12.2015	F	21Y8M	660	16	1	L	13	6(+++++), 10(++),24(++++ - +++++)
35	06.01.2016	F	17Y8M	520	21	1	L	13	14(+)
36	14.01.2016	Mk	17Y5M	520	21	1	L	13	14(++)
37	04.02.2016	F	6Y4M	510	13	2	L	13	4(++), 22(++), 27(++)
38	11.02.2016	M	3Y3M	620	1	12	L	13	4(++), 25(++)
39	23.02.2016	Mc	12Y4M	500	22	13	L	13	6(+++++), 13(+++++), 18(++++)
40	04.03.2016	Mc	22Y6M	520	9	8	L	13	27(+++++), 37(+++++)
41	03.03.2016	F	18Y6M	430	13	4	L	13	18(+++ - ++++),36(++), 38(+++)
42	19.04.2016	F	27Y8M	409	23	14	R	13	4 (++), 21(+++), 39(++++)
43	30.06.2016	Mc	16Y8M	405	8	4	L	13	4(++), 7(+++), 40(++), 41(++)
25	07.07.2016	F	16Y8M	490	19	8	R	13	4(+), 42(++)
44	01.09.2016	F	17Y8M	207	17	1	L	13	4(+), 13(++)

List of Abbreviations:

n/a = not applicable

+Sex: F= Female, M= Male, Mc= castrated male

++Age: Y = year, M = Month

*Breed: 1 = Oldenburg, 2 = Mecklenburger, 3 = Hanoverian, 4 = Sachsen-Anhaltiner, 5 = Westfalen, 6 = Polnisches warm blood, 7 = Renish german cold blood, 8 = Arabian, 9 = Warm blood, 10 = American Quarter Horse, 11 = Icelandic, 12 = Adalusian, 13 = Traber, 14 = German sport horse, 15 = Brandenburger, 16 = Haflinger, 17 = Appaloosa, 18 = Trakehner, 19 = Pony, 20 = Knabstrupper, 21 = Black Forest, 22 = Quarab, 23 = Zweibrücker

**Eye disease: 1 = Equine recurrent uveitis (ERU), 2 = Corneal ulcer, 3 = Corneal Erosion, 4 Chronic Uveitis suspected ERU, 5 = Eyelid trauma, 6 = Corneal laceration, 7 = Corneal abscess, 8 = Squamous cell carcinoma, 9 = Conjunctival mass, 10 = Sarcoid, 11 = Glaucoma, 12 = Phthisis bulbi, 13 = Corneal perforation, 14 = Bulbus trauma

***Sample site: L = Left eye, R= Right eye

#Manipulation: 1 = Vitrectomy, 2 = Keratectomy, 3 = Corneal debridement, 4 = Aqueous humor centhesis, 5 = Tumor excision, 6 = Gonderson flab, 7 = Eyelid correction, 8 = Performed transpalpebral catheter, 9 = Corneal sutured, 10 = Tarsorrhaphy, 11 = Collected tissues sample, 12 = Ciclosporin implantation, 13 = Enucleation

##Microbiota: 1 = *Staphylococcus haemolyticus*, 2= *Aerococcus* sp., 3 = Isolated unspecific species of bacteria, 4 = *Bacillus* sp., 5 = *Corynebacterium* sp., 6 = *Yeast* sp., 7 = *Pantoea sp.*, 8 = *Arthrobacter* sp., 9 = *Kocuria* sp., 10 = *Aspergillus sp.*, 11 = *Staphylococcus* sp. Intermedius group, 12 = *Leuconostoc* sp., 13 = *Streptococcus equi ssp.zooepidemicus*, 14 = *Staphylococcus aureus* (MRSA), 15 = *Zygomycetes* sp., 16 = *Streptococcus dysgalactiae* ssp. *equisimilis*, 17 = *Streptococcus ovis*, 18 = *Actinobacillus equuli*, 19 = *Fusobacterium* sp., 20 = *Bacteroides fragilis* group, 21 = *Enterococcus* sp., 22 = *Staphylococcus saprophyticus*, 23 = *Staphylococcus xylosus,* 24 = *Staphylococcus warneri*, 25 = *Staphylococcus epidermidis*, 26 = *Staphylococcus sciuri*, 27 = *Staphylococcus equorum*, 28 = *Staphylococcus caprae*, 29 = *Staphylococcus succinus*, 30 = *Streptomyces* sp., 31 = *Acinetobacter* sp., 32 = *Curtobacterium* sp., 33 = *Escherichia coli,* 34 = *Klebsiella oxytoca*, 35 = *Weissella* sp., 36 = *Pseudomonas* sp., 37 = *Rhizobium* sp., 38 = *Streptococcus mitis*, 39 = *Rothia* sp., 40 = *Staphylococcus aureus*, 41 = *Schimmelfungus* sp., 42 = *Streptomycetes* sp.

###Germ content: + = over enrichment broth, ++ = 1-2 colony, Isolated, +++ = low content, ++++ = moderate content, +++++ = high content

IV List of publications

Parts of this work have already been published in advance:

Poster 1

The 5th Leipziger Doktorandenforum in Leipzig, Germany

Title: Efficiency of Povidone Iodine (PI) as a disinfectant on ocular surface in companion animals and horses

T. Soimala[1], A. Lübke-Becker[2], B. Walther[2], JC Eule[1]

[1]Small Animal Clinic, Freie Universität, Berlin, Germany

[2]Institute of Microbiology and Epizootics, Freie Universität, Berlin, Germany

Presenting author: Mrs. Tanawan Soimala

Date and time of the session: Friday, 10th February 2017, 13.15-14.30

Time of presentation: 13.15-14.30

Poster 2

2017 ECVO Annual Scientific Meeting in Estoril, Protugal

ID number: 4071

Title: Occurrence of methicillin resistant *Staphylococcus Aureus* (MRSA) on ocular surfaces of horses undergoing ophthalmic manipulation.

T. Soimala[1], A. Lübke-Becker[2], B. Walther[2], JC Eule[1]

[1]Small Animal Clinic, Freie Universität, Berlin, Germany

[2]Institute of Microbiology and Epizootics, Freie Universität, Berlin, Germany

Presenting author: Mrs. Tanawan Soimala

Date and time of the session: Day 3 Saturday, 20th May 2017, 13.00-14.00

Time of presentation: 13.00-14.00

Oral Presentation 1

The 10th Doktorandensymposium, Berlin, Germany

Title: Antimicrobial activity of 0.2% Povidone-Iodine (PI) used for ocular surface disinfection in small animal undergoing ophthalmic manipulation

T. Soimala[1], A. Lübke-Becker[2], B. Walther[3], JC Eule[1]

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[3]Robert Koch Institute, Berlin, Germany

Presenting author: Mrs. Tanawan Soimala

Date and time of the session: Friday, 22nd September 2017, 14.00-14.30

Time of presentation: 14.20-14.30

Moderator: Mr. Gerson Motola

Oral Presentation 2

Der DVG-Vet-Congress 2017, Berlin, Germany

Title: Evaluation of the effectiveness of dilute Povidone Iodine (PI) as a disinfectant on small animal's ocular surface

T. Soimala[1], A. Lübke-Becker[2], B. Walther[2], JC Eule[1]

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[2]Institute of Microbiology and Epizootics, Freie Universität, Berlin, Germany

Presenting author: Mrs. Tanawan Soimala

Date and time of the session: Friday, 10th November 2017, 10.30-17.30

Time of presentation: 11.55-12.15

Moderator: Prof. Dr. Martin Kramer

V Acknowledgements

Firstly, I am sincerely thankful for the grant support from Prince of Songkha University, Thailand and Freie University of Berlin, Germany for this doctoral study.

Many thanks for all the educational resources and facilities from the Small Animal Clinic, Faculty of Veterinary Medicine, Freie University, Berlin and all staff.

I would like to express my sincere gratitude to my advisor Prof. Dr. Corinna Eule for the continuous support of my doctorate study and related research, for her patience, motivation, and immense knowledge. Her guidance helped me in all the time of research and writing of this thesis.

My sincere thanks also go to Dr. Antina Lübke-Becker and Dr. Birgit Walther, who provided, who gave access to the laboratory and research facilities. Without they precious support it would not be possible to conduct this research.

I would like to thank Institute for Veterinary Epidemiology and Biostatistics, Freie University Berlin, of Statistics and Mrs. Alva Rani James for impressive statistics advising and consulting.

I would like to thank Dr. Nina Peche and Mrs. Franziska Thienel for technical assistance, and Dr. Nils Cordes for comments that greatly improved the manuscript.

I would like to thank my friends for accepting nothing less than excellence from me.

Finally, I must express my very profound gratitude to my parents especially Ms. Supattra Sittiwannasak for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them. Thank you.

VI Selbstständigkeitserklärung

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbstständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen in Anspruch genommen habe.

Berlin, den 13.07.2018 Tanawan Soimala

mbvberlin mensch und buch verlag

49,90 Euro | ISBN: 978-3-86387-919-8