The bactericidal effect of two corneal crosslinking (CXL) protocols (standard vs. accelerated) on bacterial isolates associated with infectious melting keratitis in companion animals

Suter, Anja

Departement für Pferde,
Abteilung für Ophthalmologie
der Vetsuisse-Fakultät Universität Zürich
Direktor: Prof. Dr. Anton Fürst

Arbeit unter wissenschaftlicher Betreuung von
Prof. Dr. Simon Pot und Dr. Sarah Schmitt

The bactericidal effect of two Corneal Crosslinking (CXL) protocols (standard vs. accelerated) on bacterial isolates associated with infectious melting keratitis in companion animals

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Anja Suter
Tierärztin
von Suhr AG

genehmigt auf Antrag von

Prof. Dr. Simon Pot, Referent
Prof. Dr. Richard McMullen, Korreferent

2018

Table of Contents

1 Summary

Background: Corneal infections are common and potentially devastating diseases in all species. As pathogen resistance against antibiotic treatment is a growing concern, alternative treatment methods are an important focus of research. Corneal crosslinking (CXL) is a very promising oxygen radical-mediated alternative to antibiotic treatment. The main goal of this study was to assess whether a difference in bactericidal efficacy on clinical bacterial isolates could be observed between the current standard and an accelerated treatment protocol delivering the same total energy dose (5.4 J/cm^2).

Methods: Cryopreserved isolates were thawed, cultured, brought into suspension and irradiated for 30 minutes at 3mW/cm^2 (standard protocol) or 5 minutes at 18mW/cm^2 (accelerated protocol), respectively. After treatment, the bacterial suspensions were diluted and cultured overnight on agar plates. CFUs were then counted and statistically compared between groups using a linear mixed effects model.

Results: Both CXL protocols demonstrated a significant bactericidal effect on all tested isolates when compared to untreated controls. No difference between protocols was observed.

Conclusion: The accelerated CXL protocol can be recommended for empiric use in the treatment of bacterial corneal infections in veterinary species while awaiting culture results. This will facilitate immediate treatment and minimize the required anesthetic time or even obviate the need for general anesthesia.

2 Zusammenfassung

Allgemein: Infektionen der Kornea sind häufig auftretende und ernstzunehmende Probleme vieler Spezies. In Zeiten von steigenden Berichten über Antibiotikaresistenzen sollten vermehrt alternative Behandlungsmethoden in Betracht gezogen werden. Corneal Crosslinking (CXL) ist ein vielversprechendes Verfahren, das Mikroorganismen mittels Sauerstoffradikalen schädigt. Ziel dieser Studie war herauszufinden ob ein Unterschied besteht zwischen dem derzeitigen Standard- und einem beschleunigten Behandlungsprotokoll, welche die selbe Gesamt-Energiedosis (5.4 J/cm^2) abgeben, bezüglich bakterizider Wirkung gegen klinische bakterielle Isolate.

Methoden: Kryokonservierte Isolate wurden kultiviert, in Lösung gebracht und für 30 Minuten bei 3mW/cm^2 (Standard-Protokoll) beziehungsweise 5 Minuten bei 18mW/cm^2 (beschleunigtes Protokoll) bestrahlt. Nach der Behandlung wurden die Lösungen verdünnt und auf Agar-Platten kultiviert. Nach der Inkubation wurden CFU's gezählt und die Gruppen mittels eines linear mixed effects Modell verglichen.

Resultate: Beide CXL Protokolle zeigten einen signifikanten bakteriziden Effekt auf alle getesteten Isolate im Vergleich zu den unbehandelten Kontrollen. Es gab keinen Unterschied zwischen den zwei Protokollen selber.

Schlussfolgerung: Das beschleunigte CXL Protokoll kann bei septischer Keratitis bei veterinärmedizinischen Patienten eingesetzt werden, ohne dass auf die Resultate der Kultur gewartet werden muss. Dies erleichtert eine sofortige Therapie und verkürzt die Dauer der Anästhesie.

3 Introduction

3.1 Background

Corneal ulcers and erosions are a common affliction in veterinary patients. Approximately 20% (2194/12085) of all ophthalmic small animal patients and 30% (548/1858) of all ophthalmic equine patients seen in our clinic during the last 20 years presented with a corneal defect. These defects posed a threat to the integrity of the eye in 61% (1347/2194) and 70% (383/548) of these small animal and equine patients, respectively. The defects were perforated upon initial presentation or continued to progress to perforation in 21% (453/2194) and 14% (75/548) of the small animal and equine patients, respectively (Patient database 1995-2014, Section of Veterinary Ophthalmology, Vetsuisse Faculty, University of Zurich).

Although the causes differ, all species can be affected by secondary bacterial infections once the epithelial barrier is damaged. The conjunctival sac microbiota normally prevents the overgrowth of potentially pathogenic agents (1). Opportunistic microorganisms can originate from this normal ocular flora, and take advantage of a weakened ocular surface defense system.

Primary diseases of the ocular surface that weaken the cornea's anatomic barriers and physiologic defenses (low corneal sensation, quantitative and qualitative tear film deficiencies, exposure keratitis, trauma, eyelid abnormalities) can directly cause collagenolysis, but also predispose the cornea to secondary infections (2, 3). As a result, brachycephalic breeds and patients with tear film deficiencies (4) are two common patient categories that are especially susceptible to melting keratitis. In dogs with Keratoconjunctivitis sicca (KCS, dry eye syndrome), the bacteria are not washed from the cornea and conjunctiva effectively enough and therefore accumulate on these surfaces (4). Also, the normal tear film contains antibacterial peptides, proteinases and proteinase-inhibitors in a well balanced relationship. When the tear film can not be evenly distributed or the components are misbalanced, a convenient port of entry for pathogens is created (3).

Dogs, especially brachycephalic breeds, frequently present with bacterial infections of the cornea. Eyelid and eyelash abnormalities and tear film dysfunctions can have a great impact on the corneal integrity in dogs as well (2, 3). Trauma (e.g. through fights, scratch-injuries) and herpetic keratitis are common initiating events in cats (2, 5). Horse eyes are commonly affected by (micro)trauma and secondary fungal infections (2, 6, 7).

Primary infections play a relatively minor role in corneal diseases, but should be mentioned nevertheless. Putative herpes virus infection is the most common primary corneal infection and occurs far more frequently in cats than in dogs or horses. Other reported pathogens are Canine Distemper Virus, *Moraxella* spp., and *Mycoplasma* spp. (2, 3, 8). Since *Mycoplasma* spp. require specialized growth media or additional diagnostic methods, they may go undetected (8, 9).

Once present, an injury to the cornea can heal rather quickly (within hours to days), especially if the injury only affected the surface epithelium, or it can result in serious complications that might threaten eyesight or the globe itself (3).

The majority of complications are caused by secondary bacterial infections. In the course of the inflammatory process the corneal stroma may begin to melt (keratomalacia). This is a result of activated enzymes such as collagenases, elastases, matrix-metalloproteinases

(MMPs) and cathepsins and an imbalance between these proteolytic enzymes and the proteinase inhibitors present in the cornea and precorneal tear film. Such a release of collagenases is most often caused by secondary bacterial or rarely fungal infections (10). The malacic process can lead to the corneal ulcer deepening, descemetocele formation, and possibly progression to corneal perforation (2, 3, 11).

3.2 Dogs

Research on the resident microflora showed positive isolation rates of 46% to 91% in clinically healthy dogs and isolation rates of 66.2% (12) and 53% (8) in dogs with external ocular disease (conjunctivitis, traumatic laceration, blepharitis, dacryocystitis, and corneal ulceration). Variations in the type and frequency of isolates may be a result of geography, culturing technique, breed and season (11).

Previous studies concluded that gram-positive bacteria predominate the normal canine ocular microflora, with *Staphylococcus* (*S.) epidermidis*, *S. aureus*, *Streptococcus* spp., *Neisseria* spp., *Enterobacteriaceae*, and *Pseudomonas* spp. as most prevalent isolates (1, 9, 11).

In an investigation in Beijing, China, *Staphylococcus* spp. were the most frequently isolated bacteria from 29 eyes with ulcerative keratitis. *Streptococcus* spp. and *Pseudomonas* spp. were second, and third, respectively (13). Gram-positive bacteria predominated over gram-negative in the conjunctival sac of clinically normal dogs and dogs with ulcerative keratitis in Ceará, Brazil, and *Staphylococcus* spp. was the most frequently isolated genus (1, 14).

3.3 Cats

Besides primary pathogens such as Feline Herpes Virus 1 (FHV1), cats are also at risk for trauma, and secondary bacterial infections (2, 11).

In Taiwan, 51% *Staphylococcus* spp. (*S. aureus* (43.3%), *S. epidermidis* (30%), *S. warneri* (26.6%)), 13.5% *Pseudomonas (P.) aeruginosa*, 10% *Pasteurella multocida* and 8% beta-haemolytic *Streptococcus* spp. were isolated from cats with ulcerative keratitis. (15). The following rates have been reported for healthy cats: *Staphylococcus* spp. 27%, *Corynebacterium* spp. 1.3-5%, *Bacillus* spp. 3-5%, *Streptococcus* spp. 2-2.5% and *Mycoplasma* spp. 0-5% from the conjunctiva and *Staphylococcus* spp. 23-28%, *Streptococcus* spp. 0-2%, *Bacillus* spp. 2-5% and *Corynebacterium* spp. 1.6% from the eyelids (16, 17). Overall, the cat has a lower presence of bacteria on the normal ocular surfaces compared to the dog (11, 16, 18).

3.4 Horses

Horses might be more prone to ocular trauma due to their large globe and exposed position of the cornea (7). It has also been reported that thoroughbred race horses commonly suffer corneal injury due to track surface material being kicked up by other horses during a race (19). In one study the most frequently occuring bacterial isolate was *P. aeruginosa* (22%), followed by *Streptococcus equi* ssp. *zooepidemicus* (13%) and *S. aureus* (6%) (20). Another study also found *S.epidermidis* and *S. xylosus* at 6.8% each (19).

3.5 Diagnostics

The clinical evaluation of a potential ocular surface infection includes several diagnostic tests. First, a thorough examination includes slit lamp biomicroscopy of eyelid, third eyelid, corneal and conjunctival anatomy and function. A Schirmer tear test on both eyes was used to exclude KCS as a primary cause of the corneal condition. Fluorescein staining combined with a slitlamp biomicroscopy examination was performed to evaluate the extent and depth of a corneal defect. Bacterial and fungal sampling with sterile swabs and subsequent culture and sensitivity testing, and cytology were performed to evaluate the degree of inflammation and to identify potential causal microorganisms (2, 10).

3.6 Conventional therapy

Initial therapy of any case of septic corneal disease, besides identification and removal or correction of the cause, consists of topical antimicrobial therapy, a mydriatic agent and in small animals, placement of an e-collar to prevent further damage (2). Anticollagenase treatment can be added, depending on the presence of a stromal defect/melting process and/or the presence and density of a neutrophil infiltrate (2, 21, 22).

Studies have shown that corneal ulcers may heal faster and with fewer complications if anticollagenases are utilized (2, 21, 22). They help to prevent enzymatic degradation of stromal collagen and consequently reduce the progression of stromal melting, increase epithelial healing and minimize corneal scarring (2, 21, 22). Effective examples of proteinase inhibitors used in veterinary ophthalmology, among others, are disodium ethylene-diaminetetra-acetic acid (EDTA) and autologous serum (2, 21, 22).

An empiric antimicrobial choice for prophylaxis or treatment of ocular surface infections is a combination ophthalmic ointment referred to as „triple antibiotic“, which contains neomycin, polymyxin B and bacitracin (4, 23, 24). This combination provides broad-spectrum antimicrobial activity (25). While these drugs are not lipid-soluble, they penetrate into the stroma when the corneal epithelium is disrupted (25). Neomycin shows good activity against *Staphylococcus* spp. and some gram-negative bacteria, polymyxin B covers *P. aeruginosa* as well as *E. coli*, and bacitracin is active against gram-positives, similar to beta-lactam antibiotics (26). In ophthalmic solutions, bacitracin was replaced with gramicidin, another polypeptide antibiotic, due to its transcorneal penetration capability. Gramicidin has the same qualities as bacitracin; it is active against gram-positive bacteria and should not be administered systemically due to toxicities (3).

Other topically applied antibiotics used for corneal diseases in veterinary ophthalmology, especially after antimicrobial sensitivity testing, are gentamicin, chloramphenicol, tetracycline (oxytetracycline), erythromycin, tobramycin, and in severe cases where potentially resistant pathogens are involved, fluoroquinolones such as, ciprofloxacin, ofloxacin, and moxifloxacin (4, 24-27).

3.6.1 Antimicrobial resistance

As stated in our study from 2017 (23), *Streptococcus* and *Staphylococcus* spp. demonstrated an increased resistance against fluoroquinolones compared to previous reports. Over 50% of Streptococcus isolates were intermediately susceptible and a significant number of isolates were resistant to the second generation fluoroquinolones, enrofloxacin and norfloxacin.

Almost 50% (15/31) of canine Staphylococcus isolates was also resistant to these fluoroquinolones. In 2006 ,Tolar et al. (4) found 100% of Streptococci and Staphylococci to be susceptible to enrofloxacin. However, resistance might have been underestimated in this study, since intermediate results were pooled with susceptible results. Various reports in both human (28-32) and veterinary medicine (24), including the present study (23), clearly suggest increased fluoroquinolone resistance. Ofloxacin (2^{nd} generation), ciprofloxacin (2^{nd} generation) and moxifloxacin (4^{th} generation), are commonly used fluoroquinolones in veterinary ophthalmology. Because Staphylococci and Streptococci accounted for >66% of bacterial isolates, and >50% of those were intermediately susceptible or resistant to second generation fluoroquinolones, the routine first choice use of these antibiotics in corneal ulcer patients cannot be currently recommended. Bacterial resistance against 3^{rd} and 4^{th} generation fluoroquinolones is still relatively rare but can logically be expected to increase as has occurred for the 1^{st} and 2^{nd} generation fluoroquinolones (28, 32, 33). Gentamicin is typically used against Gram-negative pathogens, with special efficacy against *P. aeruginosa* (34), which is supported by the results of this study. The reported susceptibility of Gram-positive bacteria for gentamicin is variable. Gentamicin resistance was observed in 95-100% of tested Streptococcus isolates in our study (4, 12, 19, 20, 23, 34, 35). Cephalosporins have typically been effective against *Streptococcus* and *Staphylococcus* spp. Whereas cephalosporin resistance was not observed in *Streptococcus* spp. a cephalosporin resistance in approximately 20% of the tested Staphylococcus isolates from dogs was demonstrated (23). Varges et al. (24) reached similar conclusions, suggesting that cephalosporin resistance in *Staphylococcus* spp. might be an emerging problem. (36)

The same concern applies to the traditionally anti-staphylococcal antibiotic fusidic acid (37) to which a significant proportion of all tested Staphylococci in this study were resistant (23).

The intrinsic resistance of *Staphylococcus* and *Streptococcus* spp. to polymyxin B and of *Streptococcus* spp. to neomycin was confirmed with 100% of isolates being resistant in this study (23). Effectivity against Staphylococcus isolates was variable for neomycin. Resistance to neomycin and polymyxin B was observed in 16% of canine *P. aeruginosa* isolates (23). These results support the variable neomycin resistance in *Staphylococcus* spp. and *Pseudomonas* spp. strains previosly reported (4, 12). Pseudomonas isolates are routinely susceptible to polymyxin B, and acquired resistance is reportedly rare (38). Bacitracin is effective against Gram-positive bacteria, but ineffective against Gram-negative organisms, and *P. aeruginosa* is intrinsically resistant to bacitracin (39).

3.6.2 Surgical therapy

Surgical stabilization of the cornea is indicated in cases where the ulcer deepens despite ongoing medical therapy or where the integrity of the globe is already significantly compromised upon initial presentation (3, 40). Methods for surgical treatment of septic ulcers include conjunctival grafts and flaps, amniotic membrane grafting, corneoscleral and corneoconjunctival transposition, autogenous corneal grafting and corneal transplantation (3, 7). Unfortunately, surgical interventions may significantly increase scar formation and lead to vision impairment; which can be quite severe, depending on ulcer size, localisation and surgical method applied (3, 7, 41, 42).

Curriculum vitae

Vorname Name	Anja Suter
Geburtsdatum	7. Januar 1989
Geburtsort	Winterthur ZH
Nationalität	Schweiz
Heimatort	Suhr AG

2001-2007:	**1.-6. Langzeitgymnasium KZU Bülach**
Juli 2007	**Abschluss Eidgenössische Maturität**
2009-2015	**Studium der Veterinärmedizin an der Vetsuisse Fakultät Zürich, Universität Zürich, Schweiz**
Januar 2016	**Abschluss med. vet. an der Vetsuisse Fakultät Zürich, UZH, Schweiz**
März 2016 – Januar 2017	**Anfertigung der Dissertation** unter Leitung von Prof. Simon Pot am Departement für Pferde, Abteilung Ophthalmologie der Vetsuisse-Fakultät Universität Zürich Direktor Prof. Anton Fürst
Januar 2016 – Januar 2017	Tierärztin, Andreaspark-Tierärzte, Zürich, Schweiz
Februar 2017 – Januar 2018	Tierärztin Internship, Bessy's Kleintierklinik, Watt-Regensdorf, Schweiz
Februar 2018 – aktuell	Tierärztin, Marigin Zentrum für Tiermedizin, Feusisberg, Schweiz

3.7 Alternative or adjunctive treatment

An alternative or adjunctive treatment method has been proposed in the form of the so-called corneal crosslinking (CXL) procedure (43, 44). Utilizing UV-A light and riboflavin, CXL is a procedure that was developed for the treatment of keratoconus in humans, in which it arrests progressive loss of structural integrity of the corneal stroma (44, 45). Natural enzymatic cross-linking is part of the post-translational modification of collagen, which increases the biomechanical stability of the cornea and raises the resistance to enzymatic digestion (44, 45). Riboflavin (Vitamin B_2) acts as a photosensitizer when exposed to UV-A light with a wavelength at one of its absorption peaks (370nm), which results in the generation of free radicals (46-50). This process leads to free radical-induced elimination of microorganisms and apoptosis of cells and thus reduced inflammatory response in the irradiated area (46-48, 51). Additionally, riboflavin diffuses through cellular membranes and intercalates with microorganismal nucleic acids, inducing oxidative genomic damage (49, 50, 52-54). Finally, CXL leads to free radical-induced photochemical crosslinking and the formation of chemical bridges between protein residues and/or other molecules (45, 55-57). Possible indications for CXL treatment in human medicine are keratoconus (44), post LASIK keratectasia (58-60) and corneal edema and bullous keratopathy (61-65). The indications for the use of CXL have recently been expanded to include treatment for septic keratitis (45, 47, 66-76).

Efficacy has been established for photoactivated riboflavin treatment of infectious keratitis in veterinary and human patients (40, 77-84) in a procedure called photoactivated chromophore for infectious keratitis-corneal crosslinking (PACK-CXL). However, variability in efficacy between individual patients exists, especially in horses (81, 85). All authors concluded that PACK-CXL represents a very promising treatment method for infectious melting keratitis, but further investigation is required in order to optimize the energy delivery protocol in veterinary medicine (79-81, 84). Corneal crosslinking used for the specific clinical indication of infectious keratitis will be identified as PACK-CXL throughout the rest of this thesis. All other uses of corneal crosslinking will be identified using the general abbreviation CXL throughout the rest of this thesis.

PACK-CXL has a variable inhibitory effect on microorganisms in vitro, depending on the type of microorganism and pretreatment (47, 73-75). A consistent bactericidal effect has been demonstrated (40, 47, 73, 74, 86, 87) using standardised, non-ocular strains or single strains obtained from human patients (47, 73, 74). However, genetic variability between strains and isolates could affect their susceptibility to external physical and chemical stimuli (88, 89), which could explain the variability in clinical efficacy. The standard CXL protocol, developed for keratoconus therapy in human patients, was first described by Wollensak and colleagues and is often referred to as the 'Dresden protocol' (44). This protocol involves 30 minutes of saturation with riboflavin and 30 minutes of UV-A irradiation (28).

Accelerated CXL protocols were developed to decrease treatment duration. According to the Bunsen–Roscoe photochemical law of reciprocity, the effects of any photochemical reaction (in the current context, the PACK-CXL procedure) can be maintained as long as the total energy delivery is also maintained by adapting the radiation intensity delivered per unit of time (fluence) (90). This implies that the total energy delivered (5.4 J/cm^2) and the effect of CXL treatment should be similar for an irradiation of 3 mW/cm^2 for 30 min, and one of 18 mW/cm^2 for 5 min. (90).

However, the biomechanical stiffening effect is oxygen dependent and decreases with treatment duration shortening and intensity increase (91, 92). The antimicrobial effect of PACK-CXL on the other hand is oxygen-independent and not affected by shortening of the PACK-CXL procedure (75). This would shorten the duration of, or obviate the need for general anesthesia in veterinary patients.

3.8. Hypotheses and aims

Hypotheses:
The following hypotheses will be tested:

1. All bacterial isolates of veterinary relevance tested are susceptible to PACK-CXL.

2. The bactericidal effect of accelerated PACK-CXL (5 min at 18mW/cm^2) is at least equal to that of the standard protocol (30 min at 3 mW/cm^2).

Specific aims:

1. Within the scope of the Masters thesis that was performed on the topic, the prevalence of bacterial isolates from corneal ulcers obtained from dogs and cats between 2003 and 2013 was analysed. The most common and pathologically relevant isolates were identified: *Staphylococcus pseudintermedius*, *Staphylococcus aureus*, *beta-hemolytic streptococci* and *Pseudomonas aeruginosa*. This panel of pathogens will be used as sample specimens to test the bactericidal efficacy of PACK-CXL (see hypothesis 1).

2. Two PACK-CXL duration and intensity settings will be compared: standard protocol PACK-CXL (30 min at 3mW/cm^2) and accelerated PACK-CXL (5 min at 18mW/cm^2)(see hypothesis 2).

4 Materials and Methods

4.1 Preliminary trials

The basic study design idea consisted of adding a bacterial solution (with a known bacterial concentration) to corneal tissue placed in a well of a well plate, which would then be illuminated at a specific irradiance (47, 73-75, 93-96). By doing this we attempted to achieve conditions that were as close to the *in vivo* situation as possible. After treatment, we would retrieve the bacteria and count colony forming units (CFU's), comparing them to CFU's obtained from an untreated control sample (74, 75, 93, 96). In order to make the study design repeatable and to eliminate all factors that might contribute to false results, a few technical details were evaluated in preliminary trials.

4.1.1 Temperature and evaporation

Temperature measurements were conducted due to our concern of inducing a significant temperature increase in small amounts of irradiated medium, which would potentially lead to bacterial growth arrest and evaporation losses.

No temperature change occured as measured with an IR thermometer (IR Thermometer Dual Laser EXTECH INSTR. 42509, FLIR Commercial Sysems Incorporated, Nashua, USA) during the 30-minute irradiation of 30µl 0.9% Natrium Chloride solution (B. Braun Medical AG, Sempach, Switzerland) with 3mW/cm^2 irradiance (focal distance of approximately 5 cm). Virtually no fluid evaporation was detected during a 30 minute irradiation with 3mW/cm^2 irradiance as measured via fluid repipetting post CXL treatment.

4.1.2 Corneal tissue

Porcine corneal tissues were used in ten earlier trials following a modified experimental protocol. Freshly enucleated nonbrewed porcine eyes were obtained at a local slaughterhouse (Schlachthof Zürich, Veterinärdienst Stadt Zürich, Herdernstrasse 63, 8004 Zürich). The epithelium was bluntly removed with scalpel blades (Swann Morton® 10&15, Swann-Morton Limited, Sheffield, UK) and the cornea with a scleral margin was isolated. The corneas were placed on a microkeratome (SCHWIND Carriazo Pendular, Schwind eye-tech-solutions, Kleinostheim, Deutschland) and lamellae of 130µm thickness and 10 mm diameter were cut (75). To avoid contamination an antisepsis-protocol was tested. The corneal lamellae were placed into a 1:50 Betadine (Betadine Lösung standartisiert, Mundipharma Medical Company, Hamilton/Bermuda, Zweigniederlassung Basel) : 0.9%-NaCl solution for 1 minute, then dipped in sterile 0.9% NaCl and placed in another vessel with sterile 0.9% NaCl for transport to the bacteriology lab.

4.1.3 Treatment groups

ATCC strains of *P. aeruginosa* (ATCC 27853), *S. pseudintermedius* (ATCC 21284) and *Streptococcus canis* (isolate from veterinary patient with septic keratitis) were chosen for these pilot experiments. Working solutions held a bacterial concentration of $4.5x10^4/30\mu l$.

In the corneal lamella pre-trials the riboflavin/ultraviolet-light (Ri/UV) treatment group was compared to three control groups: a no treatment control group (C) – a control group that was UV-A treated only, without the presence of riboflavin in the bacterial suspension (UV) – and a control group that had riboflavin present in the bacterial suspension but was not UV-A treated (Ri).

Treatment group in pre-trials:

- Ri + UV-A

Control groups in pre-trials:

- No treatment (C)
- UV-A only
- Ri only

4.1.4 Retrieval of bacterial isolates for quantitative culture

For the experiments, the individual lamellae were placed into single wells of a 48-well cell culture plate (Falcon® Multiwell 48 well, Corning Incorporated, Corning, USA) and then covered with either 30µl 0.1% riboflavin containing bacterial suspension (Ri and Ri/UV) or 30µl 0.9%-NaCl containing bacterial suspension (C and UV). After irradiation of the Ri/UV and UV samples, the 30µl of bacterial suspension were retrieved for all four experimental groups separately, and diluted in the same way as in the main experiments. The corneal lamellae were discarded after suspension retrieval.

Preliminary experiments were performed to determine how to retrieve the bacteria remaining on the lamellae: In two trials, lamellae were pressed onto an agar plate. Since no dilution was possible for this procedure, the contact areas only showed bacterial overgrowth and quantification was not possible. Also, due to the non-rigid nature of the humid lamellae, it was not possible to perform the direct contact printing in a standardised manner.

Another trial involved rinsing solutions. The lamellae were placed in a 0.9% NaCl solution and shaken on a plate shaker at 500 rpm for 30 minutes. Afterwards, the solutions were diluted and cultivated on agar plates in the same way as in the main experiments. The results were comparable to those of the primary solution (that was retrieved after irradiation). A third method that was discussed was homogenisation of the lamellae, followed by the same methods (dilution and cultivation on agar with CFU counting). This approach was not pursued further due to ambiguity about how to homogenize the lamellae, and whether this approach itself would affect bacterial growth.

4.1.5 Conclusions

The trial using porcine lamellae was performed 5 times with *S. pseudintermedius* (ATCC 21284), 3 times with *P. aeruginosa* (ATCC 27853) and twice with *Streptococcus canis*. The results were inconsistent, even between repeats on the same bacterial strain with the same methods: In some cases less growth was observed in the control group (C) than in the treated group (Ri/UV). These results imply that the presence of the corneal tissue was somehow responsible for the large variation between measurements. This assumption was confirmed by obtaining consistently repeatable measurements within bacterial strains using the final experimental protocol outlined in the main study (see Results).

The ‘corneal lamella’ protocol did not demonstrate any benefits compared to the final experimental protocol used in the main study and was discarded due to its needlessly complicated and time-consuming design as well as the poorly reproducible outcome.

Since no differences in bacterial survival were observed between the three control groups (C, UV-A only and Ri only), the UV-A only (UV), and the riboflavin only (Ri) controls were omitted in the main experiments.

4.2 Bacterial isolates:

During a period of two years (2013-2014), 30 bacterial isolates from veterinary patients with septic keratitis that were presented to the University of Zurich Veterinary Medical Teaching Hospital were collected and cryopreserved at -80°C at the Institute for Veterinary Bacteriology (IVB), Vetsuisse Faculty, University of Zurich. In accordance with the IVB and the Section for Epidemiology 18 of the collected wild type isolates were selected to participate in

the study: dogs (11), horses (5), and cat and guinea pig (1 each) (Table 1). Inclusion criteria were that the isolate had to originate from a septic corneal defect/ulcer from a companion animal (incl. horses) patient at our clinic and had to belong to either one of the genera Staphylococcus, Streptococcus, Pseudomonas or Pasteurella, since those were the most commonly isolated bacterial pathogens from cats, dogs and horses with septic keratitis that were presented to the University of Zurich Veterinary Medical Teaching Hospital as evaluated in a previous study (23). This explains the unbalanced number of isolates per patient species and bacterial species. The goal was to select isolates that are frequently responsible for septic processes in the cornea of companion animals. More seldomly seen pathogens like *Acinetobacter* spp., *Moraxella* spp. or *Serratia* spp. were excluded as well as *Mycoplasma* spp., *Chlamydophila* spp. and *Leptospira* spp. Fresh subcultures (see below) of these frozen isolates were prepared on Columbia Sheep Blood plates (Oxoid, Pratteln, Switzerland).
The spectrum of isolates consisted of the following species:

- *Staphylococcus* spp.: 8 isolates
- *Streptococcus* spp.: 5 isolates
- *Pasteurella* spp.: 3 isolates
- *P. aeruginosa*.: 2 isolates

Table 1: List of the isolates used in this study

Isolate genus	**Case Number**	**Isolate species**	**Sampled species**
Staphylococcus	14-1547 SK1	*Staphylococcus aureus*	Horse
	15-1745 SK1	*Staphylococcus aureus*	Horse
	14-1774 SK2	*Staphylococcus epidermidis*	Dog
	15-395	*Staphylococcus epidermidis*	Dog
	15-1852 SK1	*Staphylococcus epidermidis*	Guinea Pig
	15-1913 SK5	*Staphylococcus pseudintermedius*	Dog
	15-1125 SK1	*Staphylococcus haemolyticus*	Dog
	15-1305 SK2	*Staphylococcus lentus*	Horse
Streptococcus	15-799 SK1	*Streptococcus canis*	Dog
	15-1371 SK1	*Streptococcus canis*	Dog
	15-1913 SK4	*Streptococcus canis*	Dog
	15-1305 SK1	*Streptococcus equi ssp. zooepidemicus*	Horse
	14-1547 SK1	*Streptococcus dysgalactiae ssp. equisimilis*	Horse
Pseudomonas	15-1308 SK1	*Pseudomonas aeruginosa*	Dog
	15-1670 SK1	*Pseudomonas aeruginosa*	Dog
Pasteurella	15-1353 SK1	*Pasteurella multocida*	Cat
	15-1371 SK3	*Pasteurella canis*	Dog
	16-110 SK1	*Pasteurella dagmatis*	Dog

4.3 Bacterial suspension

Each isolate (Table 1) was streaked onto a new Columbia Sheep Blood agar plate (Oxoid, Pratteln, Switzerland) and incubated over 20-24 hours at +37°C to obtain a uniform population with balanced growth. A 0.5 McFarland suspension was then prepared from these subcultures. The bacterial concentration of this suspension amounted to $1.5x10^5$/µl. Three µl of this suspension were diluted 1:10 with 0.1% riboflavin solution (treated group (Ri/UV)) resulting in a suspension with a bacterial concentration of $4.5x10^4/30\mu l$, which was used as starting suspension for further serial dilutions.

4.4 Riboflavin solution

A riboflavin concentration of 0.1% was used in all experiments. This concentration was achieved by diluting 2ml riboflavin (Vitamin B2 Streuli, Uznach, Switzerland) with 8ml 0.9% NaCl (B. Braun Medical AG, Sempach, Switzerland).

4.4 Experimental design (See also Figure 1).

30µl from the control (C) and the therapy samples (Ri/UV) were each pipetted into a single well of a 48-well-plate (Falcon® Multiwell 48 well, Corning Incorporated, Corning, USA). The well plate was shaken for 1 minute at 500 rpm and then wrapped in aluminum foil (Figure 2), leaving a window above the therapy sample (Ri/UV) in order to protect the control sample from irradiation. The wrapped plate was placed underneath the CXL-lamp at a distance where the UV-light-meter measured 3mW/cm2 and 18mW/cm2, respectively (focal distance of approximately 5 cm).(Figure 3)

Standard protocol (3mW/cm^2 for 30 minutes): the well Ri/UV was irradiated for 15 minutes with a UV-A device (UV-X^{tm} illumination system (version 1000), IROC, Switzerland) at 3mW/cm^2. The plate was then placed on a plate shaker for one minute at 500 rpm and irradiated again for another 15 minutes. After irradiation, the plate was placed on the plate shaker for another minute.

Accelerated protocol (18mW/cm^2 for 5 minutes): the well Ri/UV was irradiated for 5 minutes with a UV-A device (CCL-VARIO Cross-linking system, Peschke Trade, Switzerland) at 18mW/cm^2. After irradiation, the plate was placed on the plate shaker for one minute at 500 rpm.

After each irradiation treatment, the 30µl of irradiated Ri/UV and non-irradiated control sample solution were retrieved from the well, pipetted into separate Eppendorf tubes and diluted 1:10 with 0.9% NaCl, followed by a serial dilution of both suspensions. Certain isolates required a dilution up to 10^{-5} whereas in others a dilution of 10^{-4} was sufficient.

From the two highest dilutions, 100µl each were plated onto a Columbia Sheep Blood agar plate (Oxoid, Pratteln, Switzerland). This procedure was performed in duplicate. After 20-24 hours of incubation at +37°C, colonies that had grown over night were counted on every plate (Figure 4).

The experiment was conducted twice on different days with each isolate and each protocol.

Sample groups:

- Ri/UV (standard) sample treated with Riboflavin and 30 min UV-A Irradiation at 3mW/cm^2
- C (standard) untreated control (in NaCl)
- Ri/UV (accelerated) sample treated with Riboflavin and 5 min UV-A Irradiation at 18mW/cm^2
- C (accelerated) untreated control (in NaCl)

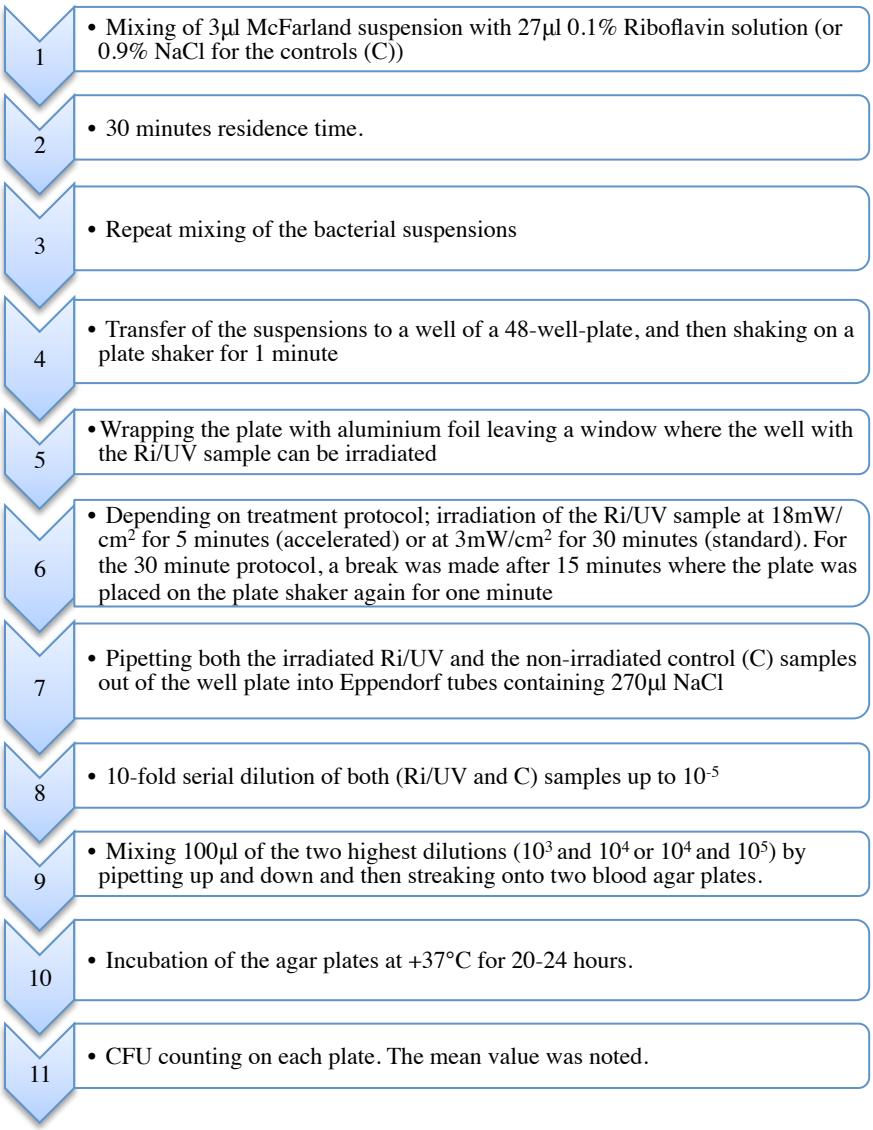

Figure 1: Test procedure

Figure 2: Well plate wrapped in aluminum foil.

Figure 3: UV-A irradiation with CXL-lamp.

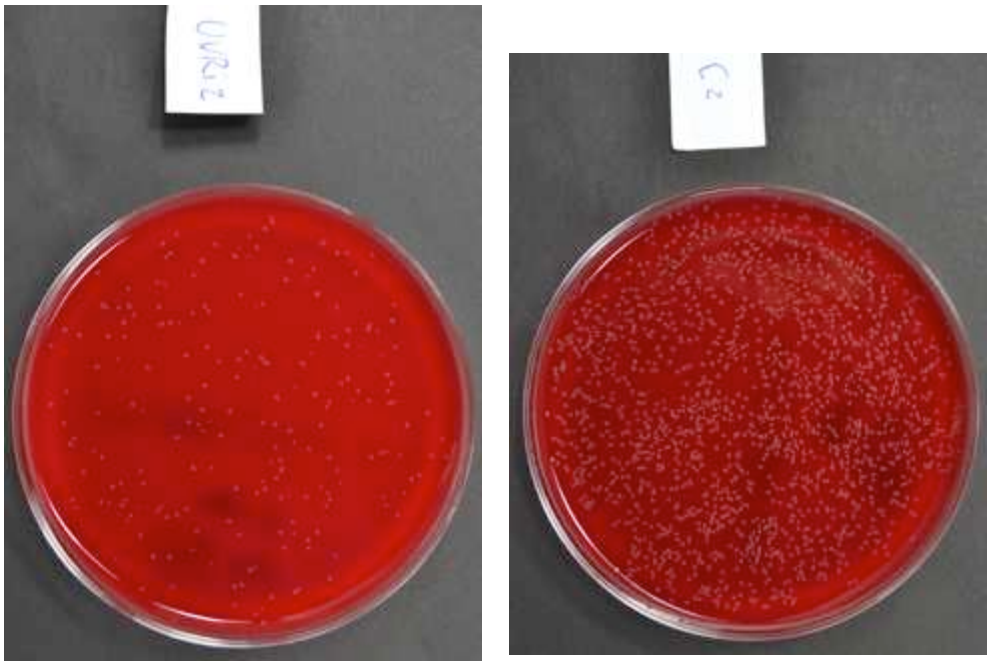

Figure 4: Representative example of agar plates after overnight incubation. Note the difference in number of bacterial colonies between the CXL treated (left: Ri/UV) and control (right: C) samples. *Pasteurella multocida* isolate. 30 minute UV-A irradiation at 3mW/cm^2.

4.6 Statistics

Mean values for all (duplicate) plates were calculated as well as the bacterial count for all of these values (Figure 5). These numbers (Tables in annex) were compared to the initial count (0.5 McFarland = $4.5x10^4/30\mu l$) in order to determine the bactericidal effect (reduction in bacterial concentration).

A linear mixed effects model was used to assess whether the combination riboflavin and UV-A (Ri/UV) and the short vs. long protocol were significantly associated with a bactericidal effect. To adjust for clustering, the isolate was considered as a random effect in the linear mixed effects model. $P < 0.05$ was considered as statistically significant.
The statistics program R 3.1.2., and the package nmle (97) were used for all statistical calculations (98)

$$c = \frac{\sum c}{n1 \times 1 + n2 \times 0{,}1} \times d$$

Figure 5: weighted average mean of colony numbers. Σc = sum of colonies of all plates, n1 = number of plates with the lowest evaluable dilution stage, n2 = number of plates with the next higher evaluable dilution stage, d = factor of the lowest evaluable dilution stage

5 Results

Bacterial concentrations are presented in the box plot diagram below (Figure 6). A significant difference in bacterial concentration was observed between the control groups and the CXL treated groups, in which the number of microorganisms was reduced by approximately 50% compared to the control groups ($p < 0.0001$). No evidence for a significant difference in bacterial concentration was observed between the standard 30-minute 3mW/cm^2 and the accelerated 5-minute 18mW/cm^2 treatment protocols ($p = 0.6$). (Table 2).

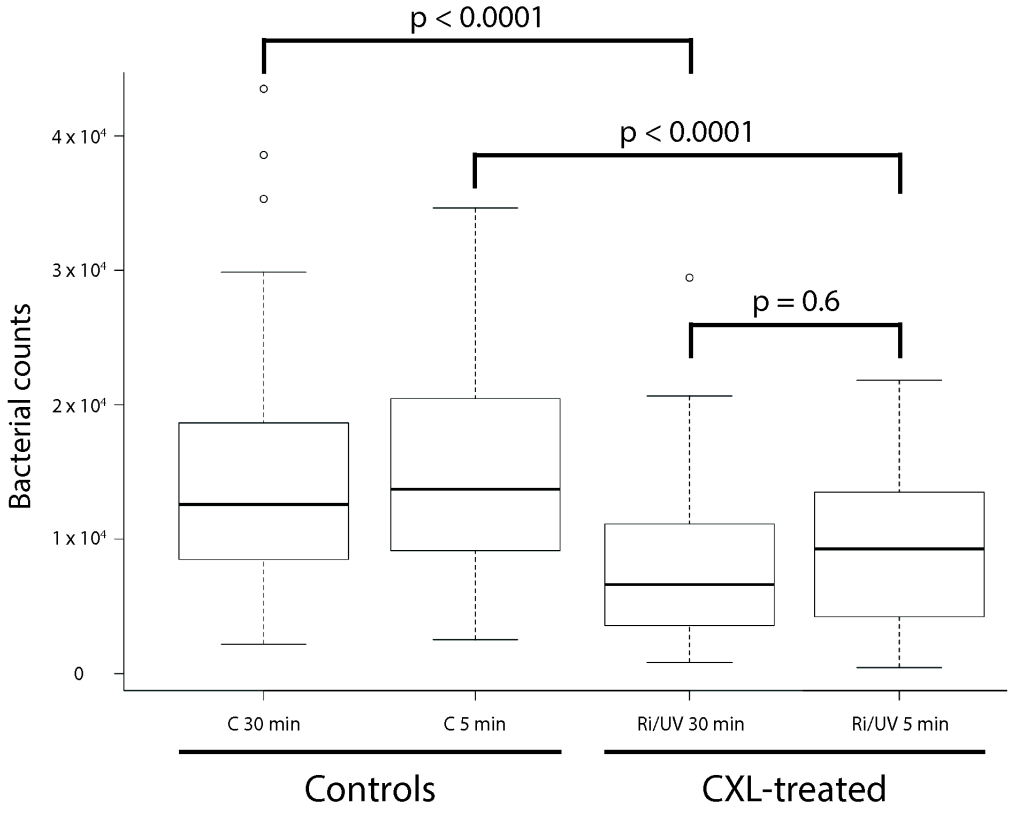

Figure 6: Box plot diagram. Comparison of two treatment protocols vs. Individual controls.

	Lower CI 95%	Bacterial concentration	Upper CI 95%	p-value
Control	11856.446	14392.209	16927.973	<0.0001
Treated group (Ri/UV)	5918.466	8479.808	11041.149	<0.0001
Concentration difference between protocols	-1592.615	573.415	2739.444	0.6013

Table 2: Effect sizes; results of the linear mixed effect model

6 Discussion

A variety of studies have demonstrated that PACK-CXL is a potentially valuable adjunctive or alternative therapy for the treatment of septic keratitis in both human and veterinary medicine (40, 78-84). The bactericidal effect of both standard 30-minute, and accelerated PACK-CXL protocols against sequenced reference strains has previously been established (75). Our study confirms these findings in non-sequenced „wild type“ isolates previously isolated from patients affected with septic keratitis in our clinic. All pathogens demonstrated equal susceptibility to both the standard and accelerated PACK-CXL protocols.

Not every bacterial species demonstrated identical susceptibility to PACK-CXL. Some isolates seemed more susceptible than others. However, a statistical difference between species could not be proven due to a lack of individual test runs (number of repeats) for each species. A higher number of test runs for every single strain in a balanced number of strains of each species must be performed before definitive conclusions about differences in sensitivity to PACK-CXL between bacterial species can be made. Richoz et al. 2014 (75) tested both standard 30-minute and accelerated PACK-CXL protocols on the two sequenced reference strains *S. aureus* SA564 and *P. aeruginosa* PA01 from human patients, and received similar killing rates for both isolates. Martins et al. (47) found CXL to be more effective against *S. epidermidis* and *S. aureus* than against *P. aeruginosa*. A heterogenous response between Staphylococcus spp. and *Pseudomonas* spp. was also reported by Makdoumi et al. (74). They suggested the following factors to have an influence on these results: Oxidative stress might have an influence on cell wall structure, intracellular transport systems and metabolic pathways, and the possibility for exposure during cell division might be increased with a shorter length of cell cycle (leading to a higher risk for errors in genetic material followed by mutations or cell death). On this account, Schrier et al. (73) proposed that CXL protocols could be tailored by using different lengths of exposure time and different excitation wavelenghts depending on the target organism. Importantly, according to Makdoumi et al. (96) antibiotic resistant strains were just as susceptible to PACK-CXL treatment when compared to non-resistant strains.

Various experimental designs were used in previous studies and include agar plates (47, 73), solutions in test tube corks (74), hand-cut porcine lamellae incubated with bacterial suspensions (75) and microscope slides with wells filled with a certain volume of fluid (96). In the present study, we decided on a bacterial suspension in 48 well plates because the diameter of each well (10mm) corresponds to the diameter of the irradiation beam (11mm). The amount of irradiated fluid was set to 30µl because this constitutes a sufficiently large volume to be handled without major pipetting losses, yet a sufficiently small volume to create a maximum layer thickness of approximately 300µm, which would most likely allow sufficient UV-A energy delivery throughout the entire fluid volume. In a pre-trial experiment we used a setting with porcine lamellae cut by a microkeratome, to ensure a defined thickness of the corneal tissue and thus optimal reproducibility. The lamellae were placed onto a cell culture plate and barely covered with a 30µl 0.1% riboflavin bacterial suspension in saline. This setup represented circumstances that were intended to be as close to real life as possible. For the final evaluations, the experimental protocol did not include the use of corneal lamellae, since no obvious differences in results were observed between protocols. Also, the

procedure involving corneal lamellae required much more preparation time and yielded results that were more difficult to reproduce.
One significant weakness of the present study could be the experimental setup. It is unclear to which depths CXL-irradiation effectivity is reached in different substrates (85, 96). Therefore, we can not conclude that the applied fluid layer thickness is appropriate for this experimental design.
The general application of PACK-CXL in veterinary medicine could mean an immense gain in the therapy of septic keratitis. A vast number of patients could be treated with less effort and financial investment, considering the complexity and high costs of a conventional conservative therapy. Shortening of the PACK-CXL treatment time would allow shorter anesthesia, and therefore reduce patient stress.
PACK-CXL shows great promise for the reduction of antibiotic administration in animals with septic keratitis. Also, due to the low asset costs of the equipment and the relatively simple application of the procedure, PACK-CXL could become a widely used treatment option for septic keratitis in private veterinary practice.

7 Acknowledgements

I would like to express my gratitude to the following contributors of this thesis:

Simon Pot: for his constant support and many ideas that contributed to the completion of this thesis.

Sarah Schmitt: for guidance and support in the laboratory, as well as constant backup and motivation.

Sabine Kling: for her technical expertise and time.

Sonja Hartnack: for her help and valuable inputs concerning the statistical part of the thesis.

Richard McMullen: for proofreading, revising, providing constructive criticism and therefore raising the scientific quality of this manuscript

Ella Hübschke: for helping me with lab work and teaching me manual skills.

Silvia Stadler, Radka Andrysikova, Christina Casola, Deborah Brütsch and Katrin Voelter as my care team for their constant moral support and crisis management.

Stiftung für Kleintiere and Schweizerische Vereinigung für Kleintiermedizin for financial support.

Special thanks also go to my **family and friends** who supported me in many ways throughout this time.

8 References

1. Prado MR, Rocha MF, Brito EH, et al. Survey of bacterial microorganisms in the conjunctival sac of clinically normal dogs and dogs with ulcerative keratitis in Fortaleza, Ceara, Brazil. Vet Ophthalmol. 2005; 8(1): 33-37.
2. Maggs DJ. Cornea and Sclera. In: Maggs DJ, Miller PE, Ofri R, Slatter DH, editors. Slatter's fundamentals of veterinary ophthalmology. 5th ed. St. Louis, Mo.: Elsevier; 2013. 184-219.
3. Ledbetter EC, Gilger BC. Diseases and Surgery of the Canine Cornea and Sclera. In: Gelatt KN, Gilger BC, Kern TJ, editors. Vet Ophthalmol. 5th ed. Ames, Iowa: Wiley-Blackwell; 2013. 976-1049.
4. Tolar EL, Hendrix DV, Rohrbach BW, et al. Evaluation of clinical characteristics and bacterial isolates in dogs with bacterial keratitis: 97 cases (1993-2003). J Am Vet Med Assoc. 2006; 228(1): 80-85.
5. Stiles J. Feline Ophthalmology. In: Gelatt KN, Gilger BC, Kern TJ, editors. Vet Ophthalmol. 5th ed. Ames, Iowa: Wiley-Blackwell; 2013. 1477-1559.
6. Gilger BC. Equine Ophthalmology. In: Gelatt KN, Gilger BC, Kern TJ, editors. Vet Ophthalmol. 5th ed. Ames, Iowa: Wiley-Blackwell; 2013. 1560-1609.
7. Brooks DE, Mattews A, Clode A. Diseases of the cornea. In: Gilger BC, editor. Equine ophthalmology. Third edition. ed. Ames, Iowa: Wiley Blackwell; 2017. 252-368.
8. Massa KL, Murphy CJ, Hartmann FA, et al. Usefulness of aerobic microbial culture and cytologic evaluation of corneal specimens in the diagnosis of infectious ulcerative keratitis in animals. J Am Vet Med Assoc. 1999; 215(11): 1671-1674.
9. Gerding PA, Jr., Kakoma I. Microbiology of the canine and feline eye. Vet Clin North Am Small Anim Pract. 1990; 20(3): 615-625.
10. Ollivier FJ. Bacterial corneal diseases in dogs and cats. Clin Tech Small Anim Pract. 2003; 18(3): 193-198.
11. Stiles J. Ocular Infections. In: Greene CE, editor. Infectious diseases of the dog and cat. 4th ed. St. Louis, Mo.: Elsevier/Saunders; 2012. 1058-1077.
12. Gerding PA, Jr., McLaughlin SA, Troop MW. Pathogenic bacteria and fungi associated with external ocular diseases in dogs: 131 cases (1981-1986). J Am Vet Med Assoc. 1988; 193(2): 242-244.
13. Wang L, Pan Q, Zhang L, et al. Investigation of bacterial microorganisms in the conjunctival sac of clinically normal dogs and dogs with ulcerative keratitis in Beijing, China. Vet Ophthalmol. 2008; 11(3): 145-149.
14. Morales A. VMAR, Salvadego M., Levy C.E. Microbiological and Clinical Aspects of Corneal Ulcers in Dogs. World Small Animal Veterinary Association World Congress Proceedings. 2009.
15. Lin CT, Petersen-Jones SM. Antibiotic susceptibility of bacteria isolated from cats with ulcerative keratitis in Taiwan. J Small Anim Pract. 2008; 49(2): 80-83.
16. Espinola MB, Lilenbaum W. Prevalence of bacteria in the conjunctival sac and on the eyelid margin of clinically normal cats. J Small Anim Pract. 1996; 37(8): 364-366.
17. Gerding PA, Cormany K, Weisiger R, et al. Survey and Topographic Distribution of Bacterial and Fungal Microorganisms in Eyes of Clinically Normal Cats. Feline Practice. 1993; 21(3): 20-23.
18. Gaskin JM. Microbiology of the canine and feline eye. Vet Clin North Am Small Anim Pract. 1980; 10(2): 303-316.
19. Wada S, Hobo S, Niwa H. Ulcerative keratitis in thoroughbred racehorses in Japan from 1997 to 2008. Vet Ophthalmol. 2010; 13(2): 99-105.

20. Sauer P, Andrew SE, Lassaline M, et al. Changes in antibiotic resistance in equine bacterial ulcerative keratitis (1991-2000): 65 horses. Vet Ophthalmol. 2003; 6(4): 309-313.
21. Ollivier FJ, Brooks DE, Kallberg ME, et al. Evaluation of various compounds to inhibit activity of matrix metalloproteinases in the tear film of horses with ulcerative keratitis. Am J Vet Res. 2003; 64(9): 1081-1087.
22. Brooks DE, Ollivier FJ. Matrix metalloproteinase inhibition in corneal ulceration. Vet Clin North Am Small Anim Pract. 2004; 34(3): 611-622.
23. Suter A, Voelter K, Hartnack S, et al. Septic keratitis in dogs, cats, and horses in Switzerland: associated bacteria and antibiotic susceptibility. Vet Ophthalmol. 2017.
24. Varges R, Penna B, Martins G, et al. Antimicrobial susceptibility of Staphylococci isolated from naturally occurring canine external ocular diseases. Vet Ophthalmol. 2009; 12(4): 216-220.
25. Dowling PM. Antimicrobial Therapy of Selected Organs; Infections of the Eyes: Conjunctivitis, Keratitis, and Endophthalmitis. In: Giguère S, Prescott, J.F., Dowling, P.M., editor. Antimicrobial therapy in veterinary medicine. 5th ed. Ames, Iowa: Blackwell Pub.; 2013. 401-405.
26. Giguère S, Prescott JF, Dowling PM. Antimicrobial therapy in veterinary medicine. 5th ed. Ames, Iowa, USA: Wiley Blackwell; 2013. xvii, 683 p. p.
27. Ledbetter EC, Hendricks LM, Riis RC, et al. In vitro fluoroquinolone susceptibility of Pseudomonas aeruginosa isolates from dogs with ulcerative keratitis. Am J Vet Res. 2007; 68(6): 638-642.
28. WHO WHO. Antimicrobial resistance: global report on surveillance. 2014.
29. Alexandrakis G, Alfonso EC, Miller D. Shifting trends in bacterial keratitis in south Florida and emerging resistance to fluoroquinolones. Ophthalmology. 2000; 107(8): 1497-1502.
30. Chalita MR, Hofling-Lima AL, Paranhos A, Jr., et al. Shifting trends in in vitro antibiotic susceptibilities for common ocular isolates during a period of 15 years. Am J Ophthalmol. 2004; 137(1): 43-51.
31. Sharma V, Sharma S, Garg P, et al. Clinical resistance of Staphylococcus keratitis to ciprofloxacin monotherapy. Indian J Ophthalmol. 2004; 52(4): 287-292.
32. Kunimoto DY, Sharma S, Garg P, et al. In vitro susceptibility of bacterial keratitis pathogens to ciprofloxacin. Emerging resistance. Ophthalmology. 1999; 106(1): 80-85.
33. Giguère SD, P.M. Fluoroquinolones. Antimicrobial Therapy in Veterinary Medicine. 5th ed. Ames, Iowa, USA: Wiley Blackwell; 2013. 295-314.
34. Dowling PM. Aminoglycosides and Aminocyclitols. Antimicrobial Therapy in Veterinary Medicine. 5th ed. Ames, Iowa, USA: Wiley Blackwell; 2013. 233-255.
35. Keller RL, Hendrix DV. Bacterial isolates and antimicrobial susceptibilities in equine bacterial ulcerative keratitis (1993--2004). Equine Vet J. 2005; 37(3): 207-211.
36. Prescott JF. Beta-lactam Antibiotics: Cephalosporins. In: Giguère S, Prescott JF, Dowling PM, editors. Antimicrobial therapy in veterinary medicine. 5th ed. Ames, Iowa, USA: Wiley Blackwell; 2013. 153-174.
37. Dowling PM. Miscellaneous Antimicrobials: Ionophores, Nitrofurans, Nitroimidazoles, Rifamycins and Others. In: Giguère S, Prescott JF, Dowling PM, editors. Antimicrobial therapy in veterinary medicine. 5th ed. Ames, Iowa, USA: Wiley Blackwell; 2013. 315-332.
38. Hariharan H, Coles M, Poole D, et al. Update on antimicrobial susceptibilities of bacterial isolates from canine and feline otitis externa. Can Vet J. 2006; 47(3): 253-255.
39. Dowling PM. Peptide Antibiotics: Polymyxins, Glycopeptides, Bacitracin and Fosfomycin. In: Giguère S, Prescott JF, Dowling PM, editors. Antimicrobial therapy in veterinary medicine. 5th ed. Ames, Iowa, USA: Wiley Blackwell; 2013. 189-198.
40. Pot SA, Gallhofer NS, Matheis FL, et al. Corneal collagen cross-linking as treatment for infectious and noninfectious corneal melting in cats and dogs: results of a prospective, nonrandomized, controlled trial. Vet Ophthalmol. 2013.
41. Hakanson N MR. Further comments on conjunctival pedicle grafting in the treatment of corneal ulcers in the dog and cat. Journal of the American Animal Hospital Association. 1988; 24: 602-605.
42. Hakanson N MR. Conjunctival pedicle grafting in the treatment of corneal ulcers in the dog and cat. Journal of the American Animal Hospital Association. 1987; 23: 641-648.
43. Wollensak G, Sporl E, Seiler T. [Treatment of keratoconus by collagen cross linking]. Ophthalmologe. 2003; 100(1): 44-49.

44. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-a-induced collagen crosslinking for the treatment of keratoconus. Am J Ophthalmol. 2003; 135(5): 620-627.
45. Spoerl E, Wollensak G, Seiler T. Increased resistance of crosslinked cornea against enzymatic digestion. Curr Eye Res. 2004; 29(1): 35-40.
46. Corbin F, 3rd. Pathogen inactivation of blood components: current status and introduction of an approach using riboflavin as a photosensitizer. Int J Hematol. 2002; 76 Suppl 2: 253-257.
47. Martins SA, Combs JC, Noguera G, et al. Antimicrobial efficacy of riboflavin/UVA combination (365 nm) in vitro for bacterial and fungal isolates: a potential new treatment for infectious keratitis. Invest Ophthalmol Vis Sci. 2008; 49(8): 3402-3408.
48. AuBuchon JP, Herschel L, Roger J, et al. Efficacy of apheresis platelets treated with riboflavin and ultraviolet light for pathogen reduction. Transfusion. 2005; 45(8): 1335-1341.
49. Goodrich RP. The use of riboflavin for the inactivation of pathogens in blood products. Vox Sang. 2000; 78 Suppl 2: 211-215.
50. Kumar V, Lockerbie O, Keil SD, et al. Riboflavin and UV-light based pathogen reduction: extent and consequence of DNA damage at the molecular level. Photochem Photobiol. 2004; 80:15-21.
51. Wollensak G, Spoerl E, Reber F, et al. Keratocyte cytotoxicity of riboflavin/UVA-treatment in vitro. Eye (Lond). 2004; 18(7): 718-722.
52. McAteer MJ, Tay-Goodrich BH, Doane S, et al. Photoinactivation of virus in packed red blood cell units using riboflavin and visible light. Transfusion. 2000; 40(10): 99s-99s.
53. Uc MH, Scott JF. Effects of ultraviolet light on the biological functions of transfer RNA. Biochem Biophys Res Commun. 1966; 22(5): 459-465.
54. Chan TC, Agarwal T, Vajpayee RB, et al. Cross-linking for microbial keratitis. Curr Opin Ophthalmol. 2016; 27(4): 348-352.
55. Wollensak G, Spoerl E, Seiler T. Stress-strain measurements of human and porcine corneas after riboflavin-ultraviolet-A-induced cross-linking. J Cataract Refract Surg. 2003; 29(9): 1780-1785.
56. Dhaliwal JS, Kaufman SC. Corneal collagen cross-linking: a confocal, electron, and light microscopy study of eye bank corneas. Cornea. 2009; 28(1): 62-67.
57. Mazzotta C, Balestrazzi A, Traversi C, et al. Treatment of progressive keratoconus by riboflavin-UVA-induced cross-linking of corneal collagen: ultrastructural analysis by Heidelberg Retinal Tomograph II in vivo confocal microscopy in humans. Cornea. 2007; 26(4): 390-397.
58. Hafezi F, Kanellopoulos J, Wiltfang R, et al. Corneal collagen crosslinking with riboflavin and ultraviolet A to treat induced keratectasia after laser in situ keratomileusis. J Cataract Refract Surg. 2007; 33(12): 2035-2040.
59. Kamburoglu G, Ertan A. Intacs implantation with sequential collagen cross-linking treatment in postoperative LASIK ectasia. J Refract Surg. 2008; 24(7): S726-729.
60. Kohlhaas M, Spoerl E, Speck A, et al. [A new treatment of keratectasia after LASIK by using collagen with riboflavin/UVA light cross-linking]. Klin Monbl Augenheilkd. 2005; 222(5): 430-436.
61. Wollensak G, Aurich H, Pham DT, et al. Hydration behavior of porcine cornea crosslinked with riboflavin and ultraviolet A. J Cataract Refract Surg. 2007; 33(3): 516-521.
62. Bottos KM, Dreyfuss JL, Regatieri CV, et al. Immunofluorescence confocal microscopy of porcine corneas following collagen cross-linking treatment with riboflavin and ultraviolet A. J Refract Surg. 2008; 24(7): S715-719.
63. Ehlers N, Hjortdal J. Riboflavin-ultraviolet light induced cross-linking in endothelial decompensation. Acta Ophthalmol. 2008; 86(5): 549-551.
64. Wollensak G, Aurich H, Wirbelauer C, et al. Potential use of riboflavin/UVA cross-linking in bullous keratopathy. Ophthalmic Res. 2009; 41(2): 114-117.
65. Krueger RR, Ramos-Esteban JC, Kanellopoulos AJ. Staged intrastromal delivery of riboflavin with UVA cross-linking in advanced bullous keratopathy: laboratory investigation and first clinical case. J Refract Surg. 2008; 24(7): S730-736.
66. Anwar HM, El-Danasoury AM, Hashem AN. Corneal collagen crosslinking in the treatment of infectious keratitis. Clin Ophthalmol. 2011; 5: 1277-1280.
67. Makdoumi K, Mortensen J, Sorkhabi O, et al. UVA-riboflavin photochemical therapy of bacterial keratitis: a pilot study. Graefes Arch Clin Exp Ophthalmol. 2012; 250(1): 95-102.
68. Makdoumi K, Mortensen J, Crafoord S. Infectious keratitis treated with corneal crosslinking. Cornea. 2010; 29(12): 1353-1358.

69. Moren H, Malmsjo M, Mortensen J, et al. Riboflavin and Ultraviolet A Collagen Crosslinking of the Cornea for the Treatment of Keratitis. Cornea. 2010; 29(1): 102-104.
70. Iseli HP, Thiel MA, Hafezi F, et al. Ultraviolet A/riboflavin corneal cross-linking for infectious keratitis associated with corneal melts. Cornea. 2008; 27(5): 590-594.
71. Micelli Ferrari T, Leozappa M, Lorusso M, et al. Escherichia coli keratitis treated with ultraviolet A/riboflavin corneal cross-linking: a case report. Eur J Ophthalmol. 2009; 19(2): 295-297.
72. Khan YA, Kashiwabuchi RT, Martins SA, et al. Riboflavin and ultraviolet light a therapy as an adjuvant treatment for medically refractive Acanthamoeba keratitis: report of 3 cases. Ophthalmology. 2011; 118(2): 324-331.
73. Schrier A, Greebel G, Attia H, et al. In vitro antimicrobial efficacy of riboflavin and ultraviolet light on Staphylococcus aureus, methicillin-resistant Staphylococcus aureus, and Pseudomonas aeruginosa. J Refract Surg. 2009; 25(9): S799-802.
74. Makdoumi K, Backman A, Mortensen J, et al. Evaluation of antibacterial efficacy of photo-activated riboflavin using ultraviolet light (UVA). Graefes Arch Clin Exp Ophthalmol. 2010; 248(2): 207-212.
75. Richoz O, Kling S, Hoogewoud F, et al. Antibacterial efficacy of accelerated photoactivated chromophore for keratitis-corneal collagen cross-linking (PACK-CXL). J Refract Surg. 2014; 30(12): 850-854.
76. Schnitzler E, Sporl E, Seiler T. [Irradiation of cornea with ultraviolet light and riboflavin administration as a new treatment for erosive corneal processes, preliminary results in four patients]. Klin Monbl Augenheilkd. 2000; 217(3): 190-193.
77. Snibson GR. Collagen cross-linking: a new treatment paradigm in corneal disease - a review. Clin Experiment Ophthalmol. 2010; 38(2): 141-153.
78. Muller L, Thiel MA, Kipfer-Kauer AI, et al. Corneal cross-linking as supplementary treatment option in melting keratitis: a case series. Klin Monbl Augenheilkd. 2012; 229(4): 411-415.
79. Famose F. Evaluation of accelerated collagen cross-linking for the treatment of melting keratitis in ten cats. Vet Ophthalmol. 2015; 18(2): 95-104.
80. Famose F. Evaluation of accelerated collagen cross-linking for the treatment of melting keratitis in eight dogs. Vet Ophthalmol. 2014; 17(5): 358-367.
81. Hellander-Edman A, Makdoumi K, Mortensen J, et al. Corneal cross-linking in 9 horses with ulcerative keratitis. BMC Vet Res. 2013; 9: 128.
82. Price MO, Tenkman LR, Schrier A, et al. Photoactivated riboflavin treatment of infectious keratitis using collagen cross-linking technology. J Refract Surg. 2012; 28(10): 706-713.
83. Said DG, Elalfy MS, Gatzioufas Z, et al. Collagen cross-linking with photoactivated riboflavin (PACK-CXL) for the treatment of advanced infectious keratitis with corneal melting. Ophthalmology. 2014; 121(7): 1377-1382.
84. Spiess BM, Pot SA, Florin M, et al. Corneal collagen cross-linking (CXL) for the treatment of melting keratitis in cats and dogs: a pilot study. Vet Ophthalmol. 2014; 17(1): 1-11.
85. Gallhoefer NS, Spiess BM, Guscetti F, et al. Penetration depth of corneal cross-linking with riboflavin and UV-A (CXL) in horses and rabbits. Vet Ophthalmol. 2016; 19(4): 275-284.
86. Berra M, Galperin G, Boscaro G, et al. Treatment of Acanthamoeba keratitis by corneal cross-linking. Cornea. 2013; 32(2): 174-178.
87. Galperin G, Berra M, Tau J, et al. Treatment of fungal keratitis from Fusarium infection by corneal cross-linking. Cornea. 2012; 31(2): 176-180.
88. Kralik P, Babak V, Dziedzinska R. Repeated cycles of chemical and physical disinfection and their influence on Mycobacterium avium subsp. paratuberculosis viability measured by propidium monoazide F57 quantitative real time PCR. Vet J. 2014; 201(3): 359-364.
89. Ermolaeva SA, Varfolomeev AF, Chernukha MY, et al. Bactericidal effects of non-thermal argon plasma in vitro, in biofilms and in the animal model of infected wounds. J Med Microbiol. 2011; 60(Pt 1): 75-83.
90. Wernli J, Schumacher S, Spoerl E, et al. The efficacy of corneal cross-linking shows a sudden decrease with very high intensity UV light and short treatment time. Invest Ophthalmol Vis Sci. 2013; 54(2): 1176-1180.
91. Richoz O, Hammer A, Tabibian D, et al. The Biomechanical Effect of Corneal Collagen Cross-Linking (CXL) With Riboflavin and UV-A is Oxygen Dependent. Transl Vis Sci Technol. 2013;2(7):6.

92. Hammer A, Richoz O, Mosquera SA, et al. Corneal Biomechanical Properties at Different Corneal Cross-Linking (CXL) IrradiancesCorneal Biomechanics at Higher UV-A Irradiances. Invest Ophthalmol Vis Sci. 2014; 55(5): 2881-2884.
93. Sun B, Li ZW, Yu HQ, et al. Evaluation of the in vitro antimicrobial properties of ultraviolet A/riboflavin mediated crosslinking on Candida albicans and Fusarium solani. Int J Ophthalmol. 2014; 7(2): 205-210.
94. Meile L. Comparison of the in vitro effect of two different corneal Crosslinking-Protocols on fungal growth [Doctoral]. Zürich: Universität Zürich; 2015.
95. Kashiwabuchi RT, Carvalho FR, Khan YA, et al. Assessment of fungal viability after long-wave ultraviolet light irradiation combined with riboflavin administration. Graefes Arch Clin Exp Ophthalmol. 2013; 251(2): 521-527.
96. Makdoumi K, Backman A. Photodynamic UVA-riboflavin bacterial elimination in antibiotic-resistant bacteria. Clin Exp Ophthalmol. 2016; 44(7): 582-586.
97. Pinheiro J BD, DebRoy S, Sarkar D and R Core Team. _nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1-128. 2016.
98. Team RDC. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.; 2008.

9 Annex

9.1 Abbreviations

ATCC	American Type Culture Collection
CFU	Colony forming unit
CSB	Columbia Sheep Blood agar plate (Oxoid, Pratteln, Switzerland)
CXL	Corneal Crosslinking
EDTA	Disodium Ethylene-Diaminetetra-Acetic Acid
FHV-1	Feline Herpes Virus 1
IVB	Institute for Veterinary Bacteriology, University of Zürich
KCS	Keratoconjunctivitis sicca
MMP	Matrix-Metalloproteinase
NaCl	Sodium Chloride
PACK-CXL	Photoactivated Chromophore for infectious Keratitis – CXL
Rpm	Revolutions per minute
Spp.	Species pluralis
UV-A	Ultraviolet A radiation
Tested Groups	
C	Control
Ri	Riboflavin only
UV	UV-A only
Ri/UV	Riboflavin and UV-A in combination

9.2 Tables main experiments: CFU and bacterial concentration

Isolate	Isolate_ID	Rep_Nr	Treatment	Dilution_1a	Dilution_1b	Dilution_1MV	Dilution_2a	Dilution_2b	Dilution_2MV	Microbes_per_30uL	Decrease_Increase_%	Date	UV-Emission	Dilution_Nun
S.epidermidis	7_S.epidermidis	1	C_30Min	78	87	82.5	13	11	12	12800	-71.55	08.11.16	3.03	384
S.epidermidis	7_S.epidermidis	1	RiUV_3mW	82	66	74	8	6	7	11000	-75.55%	08.11.16	3.03	384
S.epidermidis	7_S.epidermidis	2	C_30Min	105	122	113.5	12	11	11.5	17040	-62.13%	09.11.16	2.87	384
S.epidermidis	7_S.epidermidis	2	RiUV_3mW	75	87	81	11	6	8.5	12200	-72.85%	09.11.16	2.87	384
S.epidermidis	7_S.epidermidis	1B	C_5Min	118	125	121.5	13	12	12.5	18270	-59.40%	23.11.16	18	384
S.epidermidis	7_S.epidermidis	1B	RiUV_18mW	132	157	144.5	22	9	15.5	21816	-51.52	23.11.16	18	384
S.epidermidis	7_S.epidermidis	2B	C_5Min	249	221	235	20	18	19	34635	-23.03	07/12/16	18	384
S.epidermidis	7_S.epidermidis	2B	RiUV_18mW	146	139	142.5	17	12	14.5	21408	-52.42	07/12/16	18	384
S.epidermidis	6_S.epidermidis	1	C_30Min	93	82	87.5	7	8	7.5	12900	-71.33%	08.11.16	3.03	384
S.epidermidis	6_S.epidermidis	1	RiUV_3mW	71	73	72	11	7	9	11000	-75.55%	08.11.16	3.03	384
S.epidermidis	6_S.epidermidis	2	C_30Min	86	81	83.5	10	8	9	12610	-71.97%	10.11.16	Device defect	384
S.epidermidis	6_S.epidermidis	2	RiUV_3mW	52	49	50.5	5	3	4	7410	-83.53%	10.11.16	Device defect	384
S.epidermidis	6_S.epidermidis	1B	C_5Min	141	144	142.5	15	17	16	21613	-51.97	24.11.16	18	384
S.epidermidis	6_S.epidermidis	1B	RiUV_18mW	72	89	80.5	5	9	7	11931	-73.97	24.11.16	18	384
S.epidermidis	6_S.epidermidis	2B	C_5Min	131	147	139	12	10	11	20454	-54.55	29/11/16	18	384
S.epidermidis	6_S.epidermidis	2B	RiUV_18mW	126	98	112	7	6	6.5	16158	-64.1	29/11/16	18	384
S.epidermidis	24_S.epidermidis_MRSA+	1	C_30Min	67	69	68	6	8	7	10200	-77.33%	9.11.16	Device defect	384
S.epidermidis	24_S.epidermidis_MRSA+	1	RiUV_3mW	26	17	21.5	3	4	3.5	3408	-92.42%	9.11.16	Device defect	384
S.epidermidis	24_S.epidermidis_MRSA+	2	C_30Min	123	72	97.5	9	6	7.5	14300	-68.22%	10.11.16	Device defect	384
S.epidermidis	24_S.epidermidis_MRSA+	2	RiUV_3mW	50	64	57	8	9	8.5	8930	-80.16%	10.11.16	Device defect	384
S.epidermidis	24_S.epidermidis_MRSA+	1B	C_5Min	112	120	116	11	4	7.5	16840	-62.57	24.11.16	18	384
S.epidermidis	24_S.epidermidis_MRSA+	1B	RiUV_18mW	68	72	70	6	3	4.5	10158	-77.42	24.11.16	18	384
S.epidermidis	24_S.epidermidis_MRSA+	2B	C_5Min	100	100	100	4	5	4.5	14250	-68.33	29/11/16	18	384
S.epidermidis	24_S.epidermidis_MRSA+	2B	RiUV_18mW	97	91	94	6	4	5	13500	-70	29/11/16	18	384
S.aureus	4_S.aureus	1	C_30Min	111	116	113.5	11	6	8.5	16630	-63.04	16.11.16	Device defect	485
S.aureus	4_S.aureus	1	RiUV_3mW	64	71	67.5	8	2	5	9880	-78.04	16.11.16	Device defect	485
S.aureus	4_S.aureus	2	C_30Min	139	129	134	15	15	15	20310	-54.868	17.11.16	Device defect	485
S.aureus	4_S.aureus	2	RiUV_3mW	88	76	82	8	5	6.5	11860	-73.644	17.11.16	Device defect	485
S.aureus	4_S.aureus	1B	C_5Min	119	117	118	9	12	10.5	17522	-61	23.11.16	18	485
S.aureus	4_S.aureus	1B	RiUV_18mW	67	74	70.5	7	7	7	10568	-76.52	23.11.16	18	485
S.aureus	4_S.aureus	2B	C_5Min	137	144	140.5	20	8	14	21068	-53.19	29/11/16	18	485
S.aureus	4_S.aureus	2B	RiUV_18mW	63	54	58.5	7	7	7	8931	-80.16	29/11/16	18	485
S.aureus	23_S.aureus_MRSA+	1	C_30Min	284	292	288	25	38	31.5	43500	-3.28%	08.11.16	3.03	384
S.aureus	23_S.aureus_MRSA+	1	RiUV_3mW	140	160	150	18	15	16.5	20650	-54.11%	08.11.16	3.03	384
S.aureus	23_S.aureus_MRSA+	2	C_30Min	257	249	253	33	27	30	38580	-14.23%	10.11.16	Device defect	384
S.aureus	23_S.aureus_MRSA+	2	RiUV_3mW	201	193	197	23	15	19	29450	-34.55%	10.11.16	Device defect	384
S.aureus	23_S.aureus_MRSA+	1B	C_5Min	31	40	35.5	3	4	3.5	5318	-88.19	24.11.16	18	485
S.aureus	23_S.aureus_MRSA+	1B	RiUV_18mW	24	30	27	0	0	0	3681	-91.2	24.11.16	18	485
S.aureus	23_S.aureus_MRSA+	2B	C_5Min	46	47	46.5	3	10	6.5	7227	-83.94	29/11/16	18	485
S.aureus	23_S.aureus_MRSA+	2B	RiUV_18mW	19	21	20	5	1	3	3136	-93.03	29/11/16	18	485
S.haemolyticus	11_S.haemolyticus_MRSA+	1	C_30Min	56	57	56.5	4	11	7.5	8720	-80.62%	08.11.16	Device defect	384
S.haemolyticus	11_S.haemolyticus_MRSA+	1	RiUV_3mW	40	55	47.5	3	2	2.5	6816	-84.85%	08.11.16	Device defect	384
S.haemolyticus	11_S.haemolyticus_MRSA+	2	C_30Min	45	38	41.5	3	6	4.5	6272	-86.06%	09.11.16	2.87	384
S.haemolyticus	11_S.haemolyticus_MRSA+	2	RiUV_3mW	31	34	32.5	5	4	4.5	5043	-88.79%	09.11.16	2.87	384
S.haemolyticus	11_S.haemolyticus_MRSA+	1B	C_5Min	89	75	82	6	5	5.5	11931	-73.48	24.11.16	18	384
S.haemolyticus	11_S.haemolyticus_MRSA+	1B	RiUV_18mW	16	40	28	1	3	2	4090	-90.91	24.11.16	18	384
S.haemolyticus	11_S.haemolyticus_MRSA+	2B	C_5Min	98	84	91	8	5	6.5	13295	-70.46	29/11/16	18	384
S.haemolyticus	11_S.haemolyticus_MRSA+	2B	RiUV_18mW	65	64	64.5	9	6	7.5	9818	-78.18	29/11/16	18	384

S.lentus	**13_S.lentus**	1	C_30Min	89	70	79.5	6	8	7	11790	-73.81	16.11.16	Device defect	4&5
S.lentus	**13_S.lentus**	1	RiUV_3mW	50	59	54.5	10	5	7.5	8454	-81.22	16.11.16	Device defect	4&5
S.lentus	**13_S.lentus**	2	C_30Min	78	89	83.5	5	12	8.5	12540	-72.14	17.11.16	Device defect	4&5
S.lentus	**13_S.lentus**	2	RiUV_3mW	79	99	89	6	9	7.5	13150	-70.78	17.11.16	Device defect	4&5
S.lentus	**13_S.lentus**	1B	C_5Min	89	108	98.5	11	15	13	15204	-66.22	24.11.16	18	4&5
S.lentus	**13_S.lentus**	1B	RiUV_18mW	83	112	97.5	7	10	8.5	14454	-67.88	24.11.16	18	4&5
S.lentus	**13_S.lentus**	2B	C_5Min	93	98	95.5	8	16	12	14658	-67.42	29/11/16	18	4&5
S.lentus	**13_S.lentus**	2B	RiUV_18mW	96	100	98	11	6	8.5	14522	-67.73	29/11/16	18	4&5
S.lentus	**13_S.lentus**	3B	C_5Min	103	79	91	9	10	9.5	13704	-69.55	07/12/16	Device defect	4&5
S.lentus	**13_S.lentus**	3B	RiUV_18mW	85	89	87	6	9	7.5	12886	-71.37	07/12/16	Device defect	4&5
S.pseudintermed	**29_S.pseudintermedius**	1	C_30Min	159	140	149.5	16	9	12.5	22080	-50.93%	09.11.16	Device defect	4&5
S.pseudintermed	**29_S.pseudintermedius**	1	RiUV_3mW	90	100	95	4	10	7	13900	-69.11%	09.11.16	Device defect	4&5
S.pseudintermed	**29_S.pseudintermedius**	2	C_30Min	48	47	47.5	3	2	2.5	6818	-84.85	10/11/16	Device defect	4&5
S.pseudintermed	**29_S.pseudintermedius**	2	RiUV_3mW	32	31	31.5	1	0	0.5	4363	-90.305	10/11/16	Device defect	4&5
S.pseudintermed	**29_S.pseudintermedius**	1B	C_5Min	28	28	28	6	3	4.5	4431	-90.16	24.11.16	18	4&5
S.pseudintermed	**29_S.pseudintermedius**	1B	RiUV_18mW	22	35	28.5	3	1	2	4158	-90.76	24.11.16	18	4&5
S.pseudintermed	**29_S.pseudintermedius**	2B	C_5Min	15	21	18	0	1	0.5	2522	-94.39	07/12/16	18	4&5
S.pseudintermed	**29_S.pseudintermedius**	2B	RiUV_18mW	11	11	11	0	1	0.5	1568	-96.51	07/12/16	18	4&5
St.dysgalactiae	**3_St.dysgalactiae_equisimilis**	1	C_30Min	23	28	25.5	5	5	5	4158	-90.76	15.11.16	Device defect	3&4
St.dysgalactiae	**3_St.dysgalactiae_equisimilis**	1	RiUV_3mW	22	14	18	2	2	2	2727	-93.94	15.11.16	Device defect	3&4
St.dysgalactiae	**3_St.dysgalactiae_equisimilis**	2	C_30Min	22	27	24.5	3	5	4	3885	-91.36	17.11.16	Device defect	3&4
St.dysgalactiae	**3_St.dysgalactiae_equisimilis**	2	RiUV_3mW	16	18	17	1	2	1.5	2522	-94.39	17.11.16	Device defect	3&4
St.dysgalactiae	**3_St.dysgalactiae_equisimilis**	1B	C_5Min	56	50	53	11	4	7.5	2750	-93.88	30/11/16	18	3&4
St.dysgalactiae	**3_St.dysgalactiae_equisimilis**	1B	RiUV_18mW	35	29	32	2	0	1	450	-99	30/11/16	18	3&4
St.dysgalactiae	**3_St.dysgalactiae_equisimilis**	2B	C_5Min	108	73	90.5	7	11	9	13568	-69.85	06/12/16	18	3&4
St.dysgalactiae	**3_St.dysgalactiae_equisimilis**	2B	RiUV_18mW	52	42	47	3	4	3.5	6886	-84.7	06/12/16	18	3&4
St.canis	**8_St.canis**	1	C_30Min	217	245	231	31	25	28	35310	-21.53	15.11.16	Device defect	3&4
St.canis	**8_St.canis**	1	RiUV_3mW	112	135	123.5	3	9	6	17650	-60.77	15.11.16	Device defect	3&4
St.canis	**8_St.canis**	3	C_30Min	14	15	14.5	3	0	1.5	2181	-95.15	23.11.16	Device defect	4&5
St.canis	**8_St.canis**	3	RiUV_3mW	4	6	5	2	0	1	818	-98.18	23.11.16	Device defect	4&5
St.canis	**8_St.canis**	1B	C_5Min	21	23	22	1	2	1.5	3204	-92.88	30/11/16	18	4&5
St.canis	**8_St.canis**	1B	RiUV_18mW	15	10	12.5	0	0	0	1704	-96.21	30/11/16	18	4&5
St.canis	**8_St.canis**	2B	C_5Min	44	52	48	4	7	5.5	7293	-83.79	06/12/16	18	4&5
St.canis	**8_St.canis**	2B	RiUV_18mW	18	27	22.5	1	4	2.5	3408	-92.42	06/12/16	18	4&5
St.canis	**17_St.canis**	1	C_30Min	74	88	81	8	5	6.5	11931	-73.48	15.11.16	Device defect	3&4
St.canis	**17_St.canis**	1	RiUV_3mW	33	44	38.5	4	5	4.5	5863	-86.97	15.11.16	Device defect	3&4
St.canis	**17_St.canis**	2	C_30Min	26	23	24.5	5	3	4	3886	-91.36	17.11.16	Device defect	3&4
St.canis	**17_St.canis**	2	RiUV_3mW	14	18	16	1	1	1	2318	-94.84	17.11.16	Device defect	3&4
St.canis	**17_St.canis**	1B	C_5Min	74	58	66	1	1	1	9136	-79.7	30/11/16	18	3&4
St.canis	**17_St.canis**	1B	RiUV_18mW	35	38	36.5	0	3	1.5	5181	-88.48	30/11/16	18	3&4
St.canis	**17_St.canis**	2B	C_5Min	113	102	107.5	11	6	8.5	15818	-64.85	06/12/16	18	3&4
St.canis	**17_St.canis**	2B	RiUV_18mW	54	54	54	4	5	4.5	7977	-82.28	06/12/16	18	3&4
St.canis	**28_St.canis**	1	C_30Min	74	88	81	8	5	6.5	11931	-73.48	15.11.16	Device defect	3&4
St.canis	**28_St.canis**	1	RiUV_3mW	33	44	38.5	4	5	4.5	5863	-86.97	15.11.16	Device defect	3&4
St.canis	**28_St.canis**	2	C_30Min	103	94	98.5	8	5	6.5	14318	-68.18	22.11.16	Device defect	3&4
St.canis	**28_St.canis**	2	RiUV_3mW	35	37	36	1	0	0.5	4977	-88.94	22.11.16	Device defect	3&4
St.canis	**28_St.canis**	1B	C_5Min	175	156	165.5	22	7	14.5	24545	-45.45	30/11/16	18	3&4
St.canis	**28_St.canis**	1B	RiUV_18mW	90	91	90.5	8	8	8	13431	-70.15	31.11.16	18	3&4
St.canis	**28_St.canis**	2B	C_5Min	108	112	110	10	8	9	16227	-63.94	06/12/16	18	3&4

St.canis	**28_St.canis**	2B	RiUV_18mW	69	69	69	5	2	3.5	9885	-78.03	06/12/16	18	3&4
St.equi	**12_St.equi_ssp_zooepidemicu**	1	C_30Min	64	63	63.5	5	12	8.5	9816	-78.18	15.11.16	Device defect	3&4
St.equi	**12_St.equi_ssp_zooepidemicu**	1	RiUV_3mW	19	30	24.5	2	1	1.5	3545	-92.12	15.11.16	Device defect	3&4
St.equi	**12_St.equi_ssp_zooepidemicu**	2	C_30Min	112	117	114.5	8	11	9.5	16909	-62.42	22.11.16	Device defect	3&4
St.equi	**12_St.equi_ssp_zooepidemicu**	2	RiUV_3mW	41	40	40.5	7	3	5	6204	-86.21	22.11.16	Device defect	3&4
St.equi	**12_St.equi_ssp_zooepidemicu**	1B	C_5Min	156	150	153	17	17	17	23181	-48.48	30/11/16	18	3&4
St.equi	**12_St.equi_ssp_zooepidemicu**	1B	RiUV_18mW	114	118	116	7	10	8.5	16977	-62.28	30/11/16	18	3&4
St.equi	**12_St.equi_ssp_zooepidemicu**	2B	C_5Min	165	180	172.5	14	19	16.5	25772	-42.73	06/12/16	18	3&4
St.equi	**12_St.equi_ssp_zooepidemicu**	2B	RiUV_18mW	89	96	92.5	8	15	11.5	14181	-68.48	06/12/16	18	3&4
P.aeruginosa	**14_P.aeruginosa**	1	C_30Min	105	89	97	6	10	8	14310	-68.2	15.11.16	Device defect	4&5
P.aeruginosa	**14_P.aeruginosa**	1	RiUV_3mW	34	35	34.5	0	0	0	4704	-89.55	15.11.16	Device defect	4&5
P.aeruginosa	**14_P.aeruginosa**	2	C_30Min	53	56	54.5	11	6	8.5	8590	-80.92	22.11.16	Device defect	4&5
P.aeruginosa	**14_P.aeruginosa**	2	RiUV_3mW	46	36	41	6	6	6	6409	-85.75	22.11.16	Device defect	4&5
P.aeruginosa	**14_P.aeruginosa**	1B	C_5Min	133	159	146	14	11	12.5	21613	-51.97	01/12/16	18	4&5
P.aeruginosa	**14_P.aeruginosa**	1B	RiUV_18mW	113	116	114.5	12	9	10.5	17043	-62.12	01/12/16	18	4&5
P.aeruginosa	**14_P.aeruginosa**	2B	C_5Min	84	70	77	3	7	5	11181	-75.15	06/12/16	18	4&5
P.aeruginosa	**14_P.aeruginosa**	2B	RiUV_18mW	62	64	63	7	3	5	9272	-79.4	06/12/16	18	4&5
P.aeruginosa	**21_P.aeruginosa**	1	C_30Min	153	190	171.5	18	24	21	26250	-41.66	16.11.16	Device defect	4&5
P.aeruginosa	**21_P.aeruginosa**	1	RiUV_3mW	75	79	77	6	5	5.5	11250	-75	16.11.16	Device defect	4&5
P.aeruginosa	**21_P.aeruginosa**	2	C_30Min	32	29	30.5	1	3	2	4431	-90.15	06/12/16	Device defect	4&5
P.aeruginosa	**21_P.aeruginosa**	2	RiUV_3mW	23	20	21.5	5	1	3	3340	-92.57	06/12/16	Device defect	4&5
P.aeruginosa	**21_P.aeruginosa**	1B	C_5Min	76	57	66.5	7	9	8	10158	-77.42	01/12/16	18	4&5
P.aeruginosa	**21_P.aeruginosa**	1B	RiUV_18mW	42	58	50	3	4	3.5	7295	-83.78	01/12/16	18	4&5
P.aeruginosa	**21_P.aeruginosa**	2B	C_5Min	62	70	66	5	7	6	9818	-78.18	06/12/16	18	4&5
P.aeruginosa	**21_P.aeruginosa**	2B	RiUV_18mW	47	41	44	2	3	2.5	6340	-85.91	07/12/16	18	4&5
Past.multocida	**16_Past.multocida**	1	C_30Min	135	136	135.5	13	13	13	20250	-55	16.11.16	Device defect	4&5
Past.multocida	**16_Past.multocida**	1	RiUV_3mW	39	53	46	6	5	5.5	7022	-84.4	16.11.16	Device defect	4&5
Past.multocida	**16_Past.multocida**	2	C_30Min	190	216	203	11	21	16	29863	-33.63	23.11.16	Device defect	4&5
Past.multocida	**16_Past.multocida**	2	RiUV_3mW	67	65	66	8	10	9	10227	-77.27	23.11.16	Device defect	4&5
Past.multocida	**16_Past.multocida**	1B	C_5Min	259	250	192	18	31	33.5	30750	-31.66	01/12/16	18	4&5
Past.multocida	**16_Past.multocida**	1B	RiUV_18mW	85	92	88.5	9	11	10	13431	-70.15	01/12/16	18	4&5
Past.multocida	**16_Past.multocida**	2B	C_5Min	197	158	177.5	16	10	13	25977	-42.28	07/12/16	18	4&5
Past.multocida	**16_Past.multocida**	2B	RiUV_18mW	90	106	98	7	8	7.5	14386	-68.04	07/12/16	18	4&5
Past.canis	**18_Past.canis**	1	C_30Min	58	64	61	5	7	6	9135	-79.7	16.11.16	Device defect	3&4
Past.canis	**18_Past.canis**	1	RiUV_3mW	25	11	18	2	1	1.5	2658	-94.09	16.11.16	Device defect	3&4
Past.canis	**18_Past.canis**	2	C_30Min	152	176	164	16	17	16.5	24613	-45.31	23.11.16	Device defect	3&4
Past.canis	**18_Past.canis**	2	RiUV_3mW	100	100	100	9	7	8	14727	-67.27	23.11.16	Device defect	3&4
Past.canis	**18_Past.canis**	1B	C_5Min	80	93	86.5	3	3	3	12204	-72.88	01/12/16	18	3&4
Past.canis	**18_Past.canis**	1B	RiUV_18mW	39	26	32.5	3	4	3.5	4908	-89.1	01/12/16	18	3&4
Past.canis	**18_Past.canis**	2B	C_5Min	53	63	58	1	1	1	8054	-82.11	07/12/16	18	3&4
Past.canis	**18_Past.canis**	2B	RiUV_18mW	27	30	28.5	1	4	2.5	4227	-90.61	07/12/16	18	3&4
Past.dagmatis	**30_Past.dagmatis**	1	C_30Min	53	61	57	5	4	4.5	8385	-81.36	16.11.16	Device defect	4&5
Past.dagmatis	**30_Past.dagmatis**	1	RiUV_3mW	26	21	23.5	2	4	3	3612	-91.98	16.11.16	Device defect	4&5
Past.dagmatis	**30_Past.dagmatis**	2	C_30Min	59	34	46.5	4	4	4	6886	-84.7	23.11.16	Device defect	4&5
Past.dagmatis	**30_Past.dagmatis**	2	RiUV_3mW	10	4	7	2	2	2	1227	-97.27	23.11.16	Device defect	4&5
Past.dagmatis	**30_Past.dagmatis**	1B	C_5Min	72	73	72.5	8	10	9	11113	-75.31	01/12/16	18	4&5
Past.dagmatis	**30_Past.dagmatis**	1B	RiUV_18mW	25	35	30	1	2	1.5	4295	-90.45	01/12/16	18	4&5
Past.dagmatis	**30_Past.dagmatis**	2B	C_5Min	52	50	51	8	10	9	8181	-81.82	07/12/16	18	4&5
Past.dagmatis	**30_Past.dagmatis**	2B	RiUV_18mW	19	24	21.5	4	0	2	3204	-92.88	07/12/16	18	4&5

Curriculum vitae

Vorname Name	Anja Suter
Geburtsdatum	7. Januar 1989
Geburtsort	Winterthur ZH
Nationalität	Schweiz
Heimatort	Suhr AG

2001-2007:	**1.-6. Langzeitgymnasium KZU Bülach**
Juli 2007	**Abschluss Eidgenössische Maturität**
2009-2015	**Studium der Veterinärmedizin an der Vetsuisse Fakultät Zürich, Universität Zürich, Schweiz**
Januar 2016	**Abschluss med. vet. an der Vetsuisse Fakultät Zürich, UZH, Schweiz**
März 2016 – Januar 2017	**Anfertigung der Dissertation** unter Leitung von Prof. Simon Pot am Departement für Pferde, Abteilung Ophthalmologie der Vetsuisse-Fakultät Universität Zürich Direktor Prof. Anton Fürst
Januar 2016 – Januar 2017	Tierärztin, Andreaspark-Tierärzte, Zürich, Schweiz
Februar 2017 – Januar 2018	Tierärztin Internship, Bessy's Kleintierklinik, Watt-Regensdorf, Schweiz
Februar 2018 – aktuell	Tierärztin, Marigin Zentrum für Tiermedizin, Feusisberg, Schweiz