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Dog- to- dog bite wound management – Comparison of the antiseptic efficacy of polyhexanide and hypochlorous acid with regard to reducing the use of antibiotics: A randomized clinical trial

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ABSTRACT

Therapy of dog bite wounds often includes empirical usage of antibiotics. This prospective clinical study aimed to compare the efficacy of polyhexanide and hypochlorous acid in reducing bacterial wound bio-burden and preventing the necessity for prophylactic antibiotics and to monitor the prevalence of multidrug- resistant bacteria. Thirty-four dogs with 51 bite wounds were randomly assigned to one of the treatment groups. Wounds were surgically debrided and treated with polyhexanide or hypochlorous acid. Swabs for culturing were taken three times: before and directly after debridement and directly after lavage. Veterinary patients were further divided into post-surgery care with and without prophylactic antibiotics. Wound healing until suture removal was monitored. Data were analyzed using a generalized linear model for ordinal data. Positive bacterial culture results after the first swab were obtained in 82.4 % wounds. *Staphylococcus pseudintermedius* was overrepresented in wounds, which later on developed complications. *Neisseria* species and *streptococci* species were overrepresented in cases of delayed wound closure. In 41.2 % of wounds multi-drug resistant bacteria could be detected. No negative effect of occurrence of multi-drug resistant bacteria on wound healing was observed. None of the compared antiseptics showed a significantly better wound decontamination. No superior antiseptic was found for wound lavage. Hypochlorous acid-based antiseptics provide the practical advantage of a shorter contact time. Prophylactic antibiotics should always be considered in severe dog bite wounds and might not be needed in low grade bite wounds.

Introduction

Bite wounds are one of the most frequent cases in small animal emergencies, with contamination rates of up to 95 % at presentation (Griffin and Holt, 2001; Kalnins et al., 2021; Meyers et al., 2008; Mouro et al., 2010; Nolff et al., 2019). However, infection occurs in only 17–20 % of wounds at presentation, with the time between wounding and presentation being a risk factor (Meyers et al., 2008; Mouro et al., 2010). The microbiota of infected wounds is generally characterised by multiple bacterial species, and notably, the bacteria causing an infection are usually different from those isolated immediately after debridement (Griffin and Holt, 2001). In the available literature there is no description of specific risk factors for the development of wound healing complications in dog-to-dog bite wounds (DBW). As a result, clinicians tend to use prophylactic antibiotics, often regardless of the clinical situation presented (Kalnins et al., 2022), with the risk of further contributing to antimicrobial resistance (Abbas et al., 2024; Smith et al., 2003).

The emergence and prevalence of antibiotic resistance in bacteria is one of the major challenges of modern medicine (Abbas et al., 2024). With a rising tendency, the proportion of multidrug-resistant bacteria (MDR) in the contamination rate of DBW varies from 6 % to 19 % (Nolff et al., 2016; Nolff et al., 2019; Winter et al., 2018). These numbers emphasize the urgent need for new treatments against MDR (Abbas et al., 2024).

Due to their bactericidal effect and high therapeutic index, modern

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antiseptics- like polyhexanide (PHMB) and hypochlorous acid (HOCl)- are promising alternatives (Daeschlein, 2013). PHMB, part of the biguanide group, shows broad-spectrum antimicrobial activity against gram-positive and gram-negative bacteria, biofilm-forming bacteria, and fungi (Hübner and Kramer, 2010). Studies show that wound lavage in DBW with PHMB significantly reduces wound bioburden in DBW compared to sterile saline (Nolff et al., 2019). To date, no allergenic or mutagenic properties (Barreto et al., 2020; Koburger et al., 2010; Müller and Kramer, 2008) and no resistance to PHMB have been demonstrated (Eberlein and Assadian, 2010; Kramer et al., 2019). Moreover, PHMB may enhance wound healing by improving microcirculation, angiogenesis, epithelialization, and promoting earlier wound closure (Eberlein and Assadian, 2010; Goertz et al., 2016; Kaehn, 2010; Müller and Kramer, 2008). In veterinary medicine, its use is reported in wound dressings and skin and wound antisepsis (Banovic et al., 2013; Lee et al., 2004).

Another antiseptic solution of interest is HOCl. It rapidly inhibits bacterial and fungal growth, actively penetrates biofilms, and is therefore effective against a wide range of microorganisms (Sakarya et al., 2014; Uri et al., 2016; Wang et al., 2007). HOCl penetrates microbial cells by passive diffusion, where it oxidizes proteins, lipids, and nucleic acids, including sulfhydryl groups in bacterial enzymes, resulting in widespread cellular damage (Andrés et al., 2022; Winter et al., 2025). Its reactions with membrane lipids and proteins disrupt membrane integrity, while protein oxidation and nucleic acid chlorination inhibit protein and DNA synthesis, which ultimately lead to suppressing bacterial growth (da Cruz Nizer et al.,2020). This non-specific mode of action makes the development of antimicrobial resistance highly unlikely (Winter et al., 2025).

In medicine, HOCl/NaOCl is used at concentrations of 0.004 % each as a combination solution or $<$ 0.06 % for pure NaOCl solutions, ensuring non-toxicity (Kramer et al., 2018). These solutions are used in wound and scar treatment, for irrigation of traumatic, acute, and chronic wounds (Gold et al., 2020; Kramer et al., 2018). HOCl's anti-inflammatory and immunomodulatory efficacy is reflected in down-regulating histamine activity, minimizing mast cell degranulation by stabilizing the cell membrane and thus minimizing cytokine release, and promoting keratinocyte and fibroblast migration (Medina-Tamayo et al., 2007; Sakarya et al., 2014). Therefore, HOCl offers optimal properties to promote wound healing (Gold et al., 2020; Medina-Tamayo et al., 2007; Sakarya et al., 2014).

HOCl has been shown to effectively reduce veterinary pathogens in vitro, suggesting a potential reduction in the need for systemic antibiotics in vivo (McCaghertyx and Woods, 2018; Uri et al., 2016).

To date, no in-vivo comparative studies exist between PHMB and HOCl on lavage efficacy. This study aims to compare these antiseptics with the hypothesis that a fast and efficient reduction in bio-burden, allows a healthy organism's immune response to manage any remaining bacterial colonization without the further need of prophylactic antibiotics.

Furthermore, the prevalence of MDR in DBW is monitored and its associations with wound complications are assessed. Given the limited literature, this study is crucial for clinicians to make evidence-based decisions for effective wound treatment.

Materials and methods

This prospective study was conducted after gaining approval from the faculty's ethics commission (286–04–10–2021, 4 October 2021). Dogs admitted to the Clinic of Small Animal Surgery and Reproduction at LMU Munich (Veterinary teaching hospital, Munich, Germany) between September 2021 to March 2023 with witnessed bite wounds requiring surgery were included. Data collected included: signalment, time between injury and presentation, pre-existing disease, injury location, type and classification (Griffin and Holt, 2001; Nolff et al., 2019), signs of infection, prior treatment, bacterial bio- burden (initially, after debridement and after lavage). Wounds with purulent discharge were considered infected as per the International Consensus for wound infection in clinical practice (Wound Infection in Clinical Practice, 2008). Only dogs with a complete follow-up until suture removal were included, whereas dogs with severe systemic comorbidities were excluded from the study. Prior to surgery, the wounds were equally randomly assigned (1:1:1) to one of three parallel treatment groups: A. Polyhexanide-biguanide (ProntoVet®, B.Braun) as the control group, B. Hypochlorous Acid 0.015 % (Vetericyn VF®, Innovacyn Inc.) and C. Hypochlorous Acid 0.05 % (Acticyn®, WDT). All antiseptics are approved for use in animals. Dogs with multiple wounds had each wound randomly allocated to one of the three treatment groups. For example, a dog with four wounds had each wound assigned and analyzed separately.

After surgery, dogs were randomly and equally assigned to treatment with or without prophylactic antibiotics (1:1:1:1:1:1). Due to evidence of higher wound healing complications in the no-antibiotic group, this second randomization was later discontinued for ethical reasons; no interim analyses for efficacy were done. After immediate adjustment of protocol, dogs were only treated without prophylactic antibiotic with minor bite wounds and at the surgeon's discretion. Data on wound healing was documented during follow-up visits or through telephonic communication with referring veterinarians or animal owners on day four and ten to twelve days after surgery (suture removal). Fig. 1 provides further information on participant flow.

Wound treatment

Before surgery, dogs underwent clinic-standard laboratory tests (including blood count, serum profile, and C- reactive protein (CrP)). General anesthesia was induced and maintained as per the anesthesiologist's discretion. During surgery, dogs received crystalloid infusion. The wounds of the anesthetized dogs were covered with dry gauze to aseptically prepare the surrounding area for surgery. The skin surrounding the wounds was aseptically prepared and washed in a routine manner using iodine solution (Jodosept PVP, Vetoquinol GmbH) and disinfected using alcohol (Softasept N, B.Braun).

During the surgery microbiological swabs (sterile transport swabs, Sarstedt AG & Co), were taken at three time points to assess the bacterial bioburden. The first swab was taken prior to any debridement to obtain a baseline value. The swab was taken by evenly rolling it over the wound surface, while avoiding contact with the surrounding skin. The wounds were then surgically debrided in a routine manner and as per the surgeon's discretion: Any foreign material and necrotic tissue were removed with a curette, ensuring the preservation of tendons, nerves, vessels, and bones. Depending on the wound's appearance, wound edges were enlarged, or contaminated material was surgically excised en bloc (Dernell, 2006). After debridement, a second culture swab was obtained using the previously described method. Wounds were photo-documented with a sterile calibration ruler before and after debridement (Fig. 2). Lavage with 0.05 % HOCl, 0.015 % HOCl and PHMB was then performed using a 20 ml syringe and a buttoned cannula (Dernell, 2006; Nolff et al., 2019). The surgeon determined the lavage volume and recorded it from the syringe scale. Afterwards, sterile gauze was soaked with the solution of interest and placed in the wound to enable the required soak time of 15 min for PHMB and five minutes for HOCl (Eberlein and Assadian, 2010; Nolff et al., 2019; Sakarya et al., 2014). The gauze was then removed and the third microbiological swab was obtained from the wound surface in the described manner. The intention of taking the swabs at these two different time points was to extract the lavage effect separate from the debridement.

Monofilament resorbable suture (Monosyn, B.Braun) was used to close the wounds and monofilament non resorbable suture (Optilene, B. Braun) was used to close the skin. If dead space formation was suspected, a Penrose drain was inserted and exited at the ventral-most point adjacent to the wound. For smaller wounds, or those without dead space

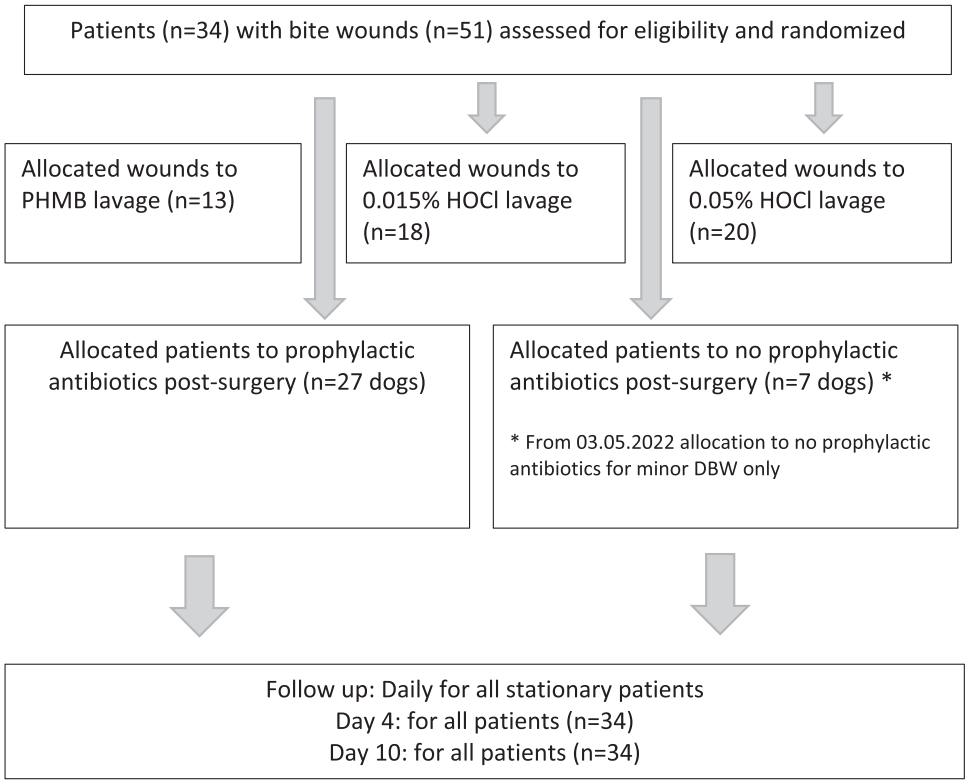

Fig. 1. Participant flow diagram.

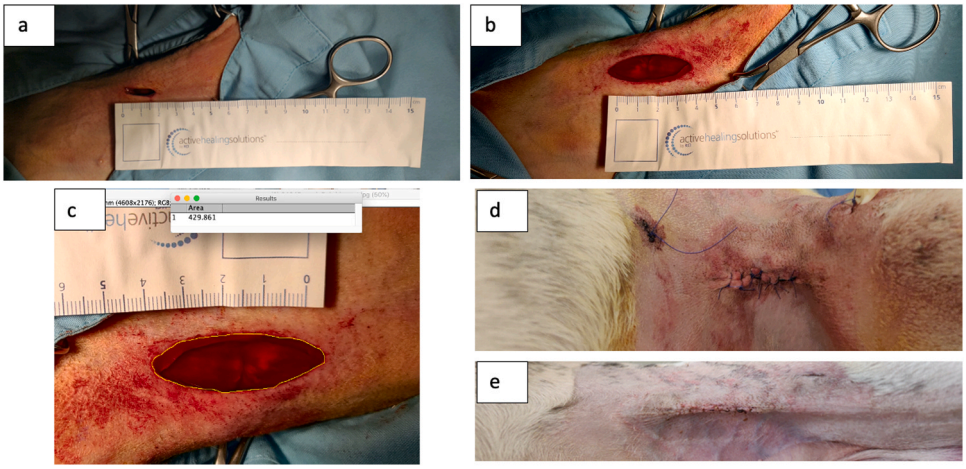

Fig. 2. Course of photo documentation, depicting one example case. a before debridement. b post debridement. c measured wound area. d wound one day post-surgery. e wound ten days post-surgery. For wound area assessment, pictures after debridement were analyzed using ImageJ freeware for digital planimetry (National Institutes of Health; http://imagej.net/ImageJ) (Aragón-Sánchez et al., 2017; Chang et al., 2011).

formation, primary closure was performed. Wounds and the drain were covered using medical adhesive drape (Cutiplast, Smith&Nephew). Surgery time (incision to removal of surgery drapes), anesthesia time (induction to extubation), lavage volume and Holt and Griffin classification were documented (Griffin and Holt, 2001; Nolff et al., 2019).

Following collection of the third swab, patients were randomly assigned to receive immediate antibiotics before antimicrobial results were available (groups A/B/C 1, amoxicillin-clavulanic acid (12.5 mg/kg i.v., Amoxiclav Hikma, Hikma Pharma GmbH) or to not receive immediate antibiotics (groups A/B/C 2). Antibiotic therapy was initiated after the third swab to avoid influence on antimicrobial test results (Bonham, 2009) and was continued orally for seven days. Patients that had received antimicrobial premedication immediately after injury from the referring veterinarians were allocated to groups A/B/C 1. The exact timing of premedication by referring veterinarians was not recorded.

In case of signs of infection or wound healing complications, another swab was taken and the antibiotic therapy was adjusted in group one and antibiotic therapy was started in group two, based on the microbiological results. Due to complications, this second randomization approach had to be revised and only dogs with non-severe bite wounds were allocated into group 2. All other dogs were automatically assigned to group 1.

Analgesia was provided based on injury severity using buprenorphine (0.01 mg/kg i.v., Buprenovet®, Animalcare Limited) alone or with carprofen (4 mg/kg i.v., Rimadyl®, Pfizer Animal Health) for three to five days.

Microbiological assessment

The diagnostic laboratory of the Institute of Microbiology, LMU Munich routinely examined the swabs by standard culturing and using maldi-TOF for bacterial species differentiation (MALDI-TOF, microflex LT, Bruker Daltonics, Bremen, Germany) and identification (Biotyper 3.1, Bruker-Daltonics, Bremen, Germany). A semi-quantitative score system was used to determine bacterial quantity for every species. The semi-quantitative scoring system categorized bacterial growth into four levels: 1: (+), indicating growth only after enrichment; 2: + , corresponding to 1–10 colony-forming units (CFU); 3: + +, corresponding to 11–100 CFU; and 4: + ++ , indicating more than 100 CFU. A summed numeric contamination score was then calculated for each time point by assigning numerical values to these categories (i.e., (+) = 1, + = 2, + + = 3, + ++ = 4) and adding the values for all detected isolates per swab. Phenotypic antibiotic resistance was assessed based on the guidelines of the Clinical and Laboratory Standards Institute (VET01) (CLSI, 2024).

Isolates were tested for susceptibility to various antibiotics (doxycycline, sulfonamide-trimethoprim, amoxicillin-clavulanic acid, cefovecin, marbofloxacin, gentamicin). For the purpose of defining multidrug resistance, isolates with intermediate results or no susceptibility were considered resistant, and only acquired antimicrobial resistance was considered (Magiorakos et al., 2012; Nolff et al., 2019). Multi-drug resistance was defined as resistance to three or more major antibiotic classes (Gandolfi-Decristophoris et al., 2013; Nolff et al., 2019). Antimicrobial test results were received after three days.

Follow- up

Hospitalized dogs received daily clinical examinations, assessing vital and wound healing parameters (swelling, redness, heat, and discharge). Hospitalization extended until drains were removed on the third postoperative day or earlier if no discharge occurred within 12 h after drape exchange (Nolff et al., 2019). Dogs with primarily closed wounds were discharged on the day of surgery. All dogs underwent a check-up (documenting vital and wound healing parameters) on day four after surgery or their day of discharge. Lab results were followed up on only if deemed necessary. Wound healing was tracked by monitoring major (pus, fever, leukocytosis) and minor signs (erythema, oedema, subcutaneous emphysema, tissue necrosis, and malodor) of infection (Mouro et al., 2010) to determine the occurrence of a local infection and delays in wound healing. A clinical infection was defined by one major or three minor signs of infection (Mouro et al., 2010). A final check was conducted at scheduled suture removal ten to twelve days after surgery, either in the clinic or by the referring veterinarian, who was then asked to provide the medical report. Complete wound closure was defined as full re-epithelialization of the wound at this time point. In cases where complications during the healing process delayed re-epithelization, the wound was documented as not completely healed by the time of suture removal. All complications were recorded and graded as minor (no intervention needed) or major (surgical intervention needed) (Nolff et al., 2019).

Statistical methods

The minimum sample size was determined utilizing polyhexanide decontamination outcomes as a reference and calculated using BIAS software for Windows Version 11.01 (Nolff et al., 2019). Randomization lists were generated for each wound location (Head/neck, trunk, extremities) using GraphPad QuickCalc's website: http://www.graphpad.com/quickcalcs/ConfInterval1.cfm (accessed August 2021) (QuickCalc, 2021). The encrypted list was maintained by independent researchers (blinded investigators), assuring allocation concealment. IBM SPSS® Statistics 27.0 was used for statistical analysis. Descriptive statistics were obtained and all data was tested for normality using the Kolmogorov-Smirnov test. As most of the metric data were not normally distributed, the Mann-Whitney *U* test was used. For more than two groups, the Kruskal-Wallis test with Bonferroni-adjusted post hoc tests was used to statistically compare the metric data. The chi- squared test was used to analyze categorical data and, for small case numbers, the Fisher's exact test.

To assess the overall decontamination efficacy, the difference of contamination scores was compared (e.g., Score 1 - Score 2 = decontamination effect by debridement; Score 2 - Score 3 = decontamination by lavage). Intra- and intergroup analyses of decontamination for each treatment were compared using a generalized linear model for ordinal data. In the generalized linear model, the individual dogs were set as subjects. The different time points reflect the dependency of the data as repeated measurements and were included as a fixed effect. The various methods of wound disinfection and grades of wound classification were set as predictors. The model accounts for data if there is a missing time point, allowing inclusion of cases with partially missing values.

The primary analysis was intention-to-treat and involved all randomly assigned wounds. Therefore, adjustments or changes in therapy during wound healing did not impact the analysis in the assigned group. The primary outcome measure focused on the proportion of wounds achieving a decontamination score reduction in order to compare the antiseptic efficacy in between groups, with additional analyses addressing wound healing outcomes and bacterial groups involved.

Results

Canine patient data

A total of 34 dogs were included in the present study. Most dogs showed one wound (25/34), followed by six with two wounds and one dog each showed three, five and six wounds, respectively. In total 51 wounds were randomly allocated to polyhexanide (PHMB) (n = 13), 0.015 % hypochlorous acid (HOCl) (n = 18) and 0.05 % HOCl (n = 20) treatment.

Of the dogs that did not receive immediate antibiotic treatment, two were assigned to the PHMB group, one to the 0.015 % HOCl group, and four to the 0.05 % HOCl group.

Dogs had a mean age of 8 years (±3.8 years) and a mean weight of 18.8 kg (±12.3 kg) at presentation. Males were predominant (21/34), with 66 % being intact.

Wound data

Wounds on trunk (20/51, 2/20 perforating the abdomen) and head/neck (19/51) were most common, followed by wounds on extremities (12/51).

Most dogs (26/34) were presented immediately after bite injury. Twenty-one dogs received treatment within 8 h of wounding, five between 8 and 24 h after wounding, and eight were treated more than 24 h after wounding. The time to treatment was not associated with the occurrence of complications (<8 h $P = 0.30$; 8–24 h $P = 1.00$; >24 h $P = 0.30$). At presentation 5/34 dogs were premedicated with an opioid (4/34 methadone; 1/34 buprenorphine). Premedication with amoxicillin-clavulanic acid was noted in 9/34 (26.5 %) of patients and could not be linked to the presence of multidrug- resistant bacteria ($P = 0.88$), to a resistance to amoxicillin-clavulanic acid ($P = 0.81$) or to the occurrence of complications ($P = 0.40$). Further premedication included NSAIDs (6/34). Majority of wounds (31/51) were treated according to Bergmann; 19/51 according to Friedrich (58 % of these were located at the head and deck) and 1/51 required negative pressure wound therapy (Nolff et al., 2019; Winter et al., 2018). No statistically significant connection was found between complications and the type of surgery performed ($P = 0.65$). Two wounds without a drain developed a complication, and 6/31 wounds with a drain developed complications. The occurrence of complications could not be associated to wound

location ($P = 0.11$) or to individuals with a single wound versus multiple wounds ($P = 1.00$). Further details for baseline values are given in Table 1. Supplementary Table 3 provides a detailed overview of baseline parameters for each wound, including time from injury to presentation, antibiotic pretreatment, and intraoperative administration of prophylactic antibiotic.

Culture results

In 82 % of wounds (42/51), the first swab yielded positive bacterial growth. In the second swab 60 % (31/51) of all samples were positive. Followed by 49 % (25/51) positive samples in the third swab in all the groups. For two wounds no second swab was taken and for one wound no third swab was taken. After a positive first swab, there was a negative second swab in 25 % (10/40) and a negative third swab in 44 % (18/41). Negative first swabs remained negative in 78 % (7/9) of cases. In 22 % (2/9) of cases there was a positive third swab after negative first swab. Table 2 provides an overview on the overall contamination at different measure points.

A significant decrease in the contamination score was observed over the course of the three measurement points ($P < 0.001$) (Fig. 3). After initial debridement there was no statistically significant difference in reduction of contamination score between the three groups (Fig. 4).

After lavage, there was no increase in the bacterial count with PHMB (0/11), but an increase in the bacterial count with both HOCl solutions in 20 % of cases. In all groups, the bacterial count remained unchanged in approximately 40 % of the cases (46 % (5/11) PHMB, 33 % (6/18) 0.015 % HOCl and 40 % (8/20) 0.05 % HOCl). PHMB achieved a decrease in bacterial count in 55 % (6/11) of cases, while HOCl solutions achieved a decrease in bacterial count in 44 % (8/18, 0.015 %) and 40 % (8/20, 0.05 %), respectively (Fig. 5).

For no single treatment group there was a significant decrease of the summed wound contamination score over time ($P = 0.585$). Intergroup comparisons between PHMB versus 0.015 % HOCl, PHMB versus 0.05 % HOCl and 0.05 % HOCl versus 0.015 % HOCl showed no significant differences in decontamination efficacy (Figs. 4 and 5). No one agent showed a significantly better decontamination efficacy in any one of the isolated bacterial groups.

Direct comparisons of wound decontamination in individuals with multiple wounds showed no significant difference between treatment groups. Fig. 6 displays the direct comparison of decontamination efficacy of 0.015 % HOCl (n = 3 wounds) vs 0.05 % HOCl (n = 3 wounds) in the one dog with six wounds.

Table 1
Overview of treatment parameters and baseline values of the different groups.

Treatment Modality	Polyhexanide	0.015 % HOCl	0.05 % HOCl
Number of wounds included (n)	13	18	20
Wound location (n):			
Trunk (20)	8/20 (40 %)	7/20 (35 %)	5/20 (25 %)
Head/neck (19)	3/19 (16 %)	8/19 (42 %)	8/19 (42 %)
Extremities (12)	2/12 (17 %)	3/12 (25 %)	7/12 (58 %)
Number of wounds with no prophylactic antibiotic (n)	2	1	4
Mean anesthesia time in minutes (sd)	106 (±69)	101 (± 68)	114 (±72)
Mean wound area in mm^2 (sd)	970 (±969,62)	1261 (±2076)	1429 (±1482)
Wound classification (Griffin and Holt, 2001)			
Grade 1 (n)	1/13 (8 %)	0	0
Grade 2 (n)	1/13 (8 %)	7/18 (39 %)	8/20 (40 %)
Grade 3 (n)	1/13 (8 %)	4/18 (22 %)	5/20 (25 %)
Grade 4 (n)	10/13 (77 %)	7/18 (39 %)	7/20 (35 %)

Table 2
Number of swabs with no bacterial growth at different time points: Swab 1 before debridement, swab 2 after debridement, swab 3 after lavage.

	Polyhexanide	0.015 % HOCl	0.05 % HOCl
Swab 1	15 % (2/13)	22 % (4/18)	15 % (3/20)
Swab 2	54 % (7/13)	33 % (6/18)	35 % (7/20)
Swab 3	84 % (11/13)	39 % (7/18)	40 % (8/20)

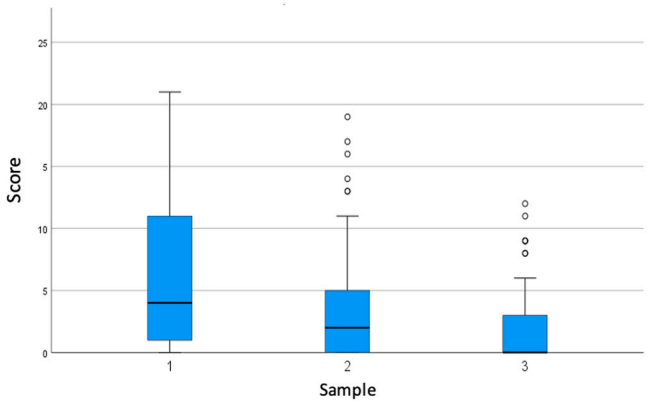

Fig. 3. Overall contamination scores at different time points. 1 before debridement, 2 after debridement, 3 after antiseptic lavage.

A total of 67 different bacterial species were isolated. The most frequently detected bacterial genera were: *Staphylococcus* (n = 51); *Pasteurella* (n = 47); *Streptococcus* (n = 32); *Neisseria* (n = 29); *Enterobacter* (n = 26); *Enterococcus* (n = 19); *Lactococcus* (n = 13); *Bacillus* (n = 11); *Clostridium* (n = 8); *Corynebacterium* (n = 5); *Pseudomonas* species (n = 4); *Acinetobacter* (n = 3); others (n = 17). Supplementary Table 1 display the distribution of isolated bacteria at different time points and Table 3 provides an overview of bacterial patterns in dogs with multiple wounds.

Complications

Minor complications were recorded in five wounds and major complications in four. Two of those wounds eventually required negative pressure wound therapy. One of the wounds with minor complications developed a major complication later on (overall complications 8/51). No veterinary patients died. Two patients with major complications had positive culture results at the time of revision. In one wound *Staphylococcus pseudintermedius* and *Streptococcus dysgalactiae* were detected, while in the other *Enterobacter cloacae* was identified. Notably, both wounds had three negative culture results initially. Table 4 provides an overview of the occurrence of complications and Tables 5 and 6 provide an overview of the different bacterial species isolated in these wounds.

Staphylococcus pseudintermedius was detected in 3/5 wounds with minor complications (first swab, $P = 0.144$) and in 3/4 wounds with major complications (first swab, $P = 0.071$). When considering the overall complication rate (combining minor and major complications), *Staphylococcus pseudintermedius* was found in 5/8 wounds (first swab, $P = 0.039$). For the bacterial group analysis, *Streptococcus* species and *Pasteurella* species were identified in 75 % (6/8) of all wounds with complications (first swab). Among wounds with minor complications both bacterial groups were detected in 4/5 wounds (*Streptococci* $P = 0.017$; *Pasteurella* species $P = 0.058$). Among major complications *Pasteurella* species were detected in 2/4 wounds and *Streptococci* in 3/4 wounds. Complications occurred in 43 % (6/14) of wounds with detected *Streptococci* ($P = 0.003$) and in 32 % (6/19) of wounds with

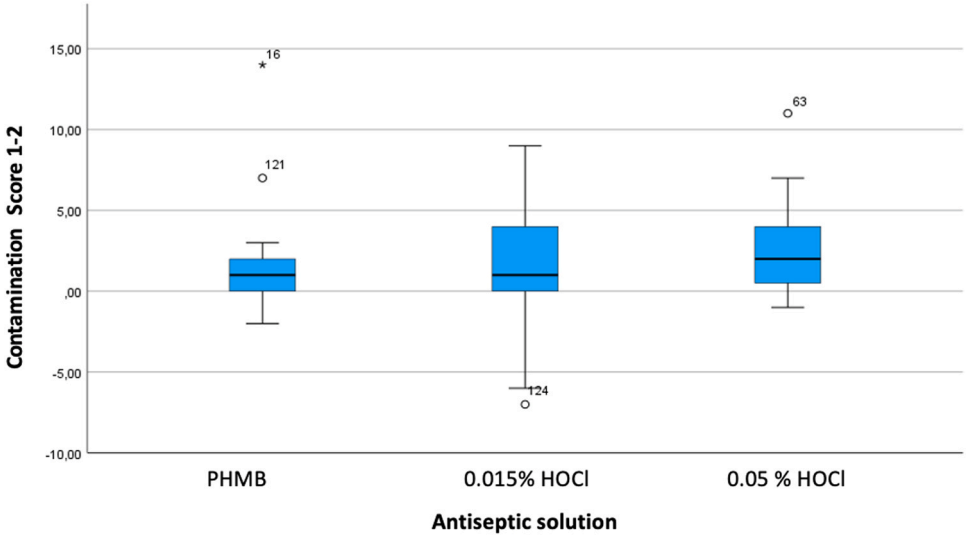

Fig. 4. Decontamination effect after debridement in all tested groups. Values > 0 show a reduction in contamination score, values < 0 show an increase in contamination score, values = 0 show no change in contamination score.

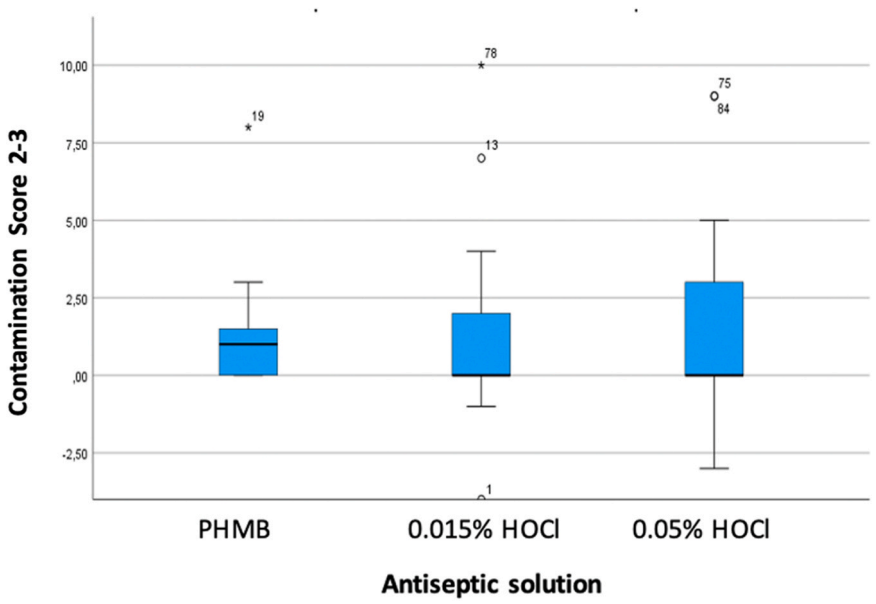

Fig. 5. Decontamination effect after lavage in all tested groups. Values > 0 show a reduction in contamination score, values < 0 show an increase in contamination score, values = 0 show no change in contamination score.

detected *Pasteurella* species ($P = 0.04$).

Incomplete wound closure at day ten occurred in 36 % (6/19) of wounds with *Streptococci* (5/14, $P = 0.013$), representing 71 % (5/7) of all wounds without complete closure at that time point. For *Neisseria* species, 36 % (4/11 wounds) showed no closure at day ten ($P = 0.031$), representing 57 % (4/7) of all wounds without complete closure on day ten.

Eight dogs (8/34) received a change in the antimicrobial treatment according to microbiological test results. In 3/8 dogs, each with one wound, the antibiotic therapy was initiated using amoxicillin-clavulanic acid (12.5 mg/kg). Amoxicillin-clavulanic acid was effective against all bacteria identified in the first swabs. For two of these wounds, microbiological testing after the third swab yielded negative results, while one wound tested positive for amoxicillin-clavulanic acid-sensitive *Staphylococcus pseudintermedius*, *Neisseria dumansiana*, and *Pasteurella canis*. In 4/8 dogs (with 1, 2, 5 and 6 wounds, respectively) marbofloxacin (2 mg/kg, Marbocyl FD 1 %®, Vetoquinol) was added as a second antibiotic to the antimicrobial treatment and in 1/8 metronidazole (15 mg/kg, Metrobactin®, Dechra) was added as a second antibiotic to the antimicrobial treatment.

Multidrug resistant bacteria (MDR)

In 41 % (21/51) of the wounds by a total of 14 dogs, a MDR could be detected at one or more time points. Nine of these dogs had one wound each. In three dogs with two wounds each, a MDR was detected in one case in both wounds and in two cases in one of the wounds. Finally, one dog with five wounds showed MDR in two of its wounds and the dog with six wounds showed MDR in all of its wounds. MDR were more frequently isolated on the extremities (9/12) than other locations (trunk 3/20, head or neck 9/19) ($P = 0.003$). Table 7 presents the distribution of MDR.

On day five therapy for wounds with MDR got adapted significantly more often compared to wounds without MDR (7/21 with MDR vs. 0/30

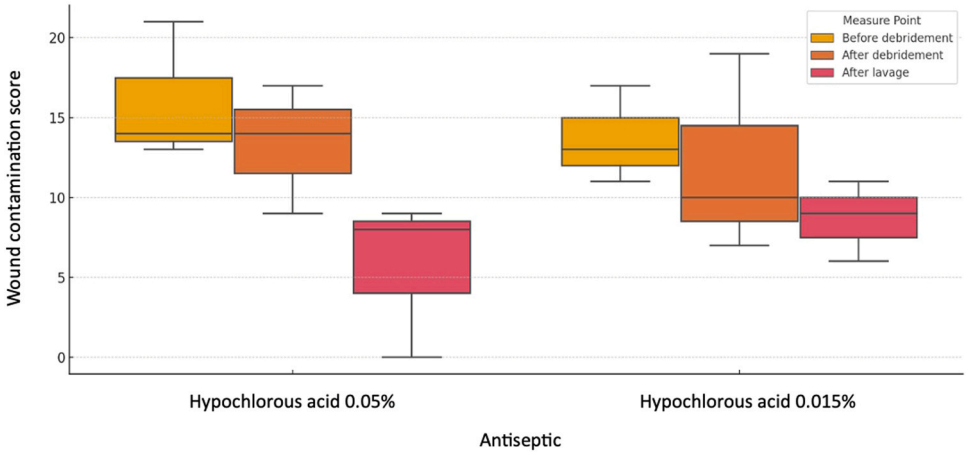

Fig. 6. Direct comparison of decontamination efficacy of 0.05 % HOCl (n = 3) vs 0.015 % HOCl (n = 3) in one individual with six wounds.

Table 3
Overview of microbiological test results in dogs with two, three and more wounds.

	Two wounds	Three wounds	> 3 wounds
Identical microbiological results	2	0	0
Overlapping microbiological results	2	1	0
Non- identical microbiological results	2	0	2

Table 4
Overview on complication occurrence.

	Polyhexanide	0.015 % HOCl	0.05 % HOCl
Complete wound closure day ten post-surgery (n)	12/13 (92 %)	16/18 (89 %)	16/20 (80 %)
Minor complications	1/13	2/18	2/20
Major complications:			
Wound infection (n)	0/13	0/18	2/20
Necrosis (n)	1/13	0/18	1/20

Table 5
Distribution of bacterial isolates from wounds, which developed minor complications (n = 5). The summed score displays the number of times each bacterium was isolated from a wound at each measurement point: Sum 1 before debridement, Sum 2 after debridement, Sum 3 after lavage.

Bacteria	Sum 1	Sum 2	Sum 3
Bacillus species	0	1	1
Corynebacterium sp.	1	0	0
Enterococcus faecalis	1	0	0
Frederiksenia canicola	4	1	0
Gram-positive (unidentified)	0	1	0
Neisseria animaloris	1	0	0
Neisseria dumasiana	2	1	1
Neisseria weaveri	2	2	0
Pasteurella canis	1	2	2
Pasteurella dagmatis	1	1	0
Pasteurella stomatis	0	1	0
Streptococcus canis	1	0	1
Streptococcus minor	1	0	0
Staphylococcus pseudintermedius	3	2	2
Stenotrophomonas maltophilia	1	0	0
Total	19	12	7

Table 6
Distribution of bacterial isolates from wounds, which developed major complications (n = 4). The summed score displays the number of times each bacterium was isolated from a wound at each measurement point: Sum 1 before debridement, Sum 2 after debridement, Sum 3 after lavage.

Bacteria	Sum 1	Sum 2	Sum 3
Frederiksenia canicola	3	1	1
Alpha- hemolytic streptococci	1	0	0
Macrococcus caseolyticus	1	0	0
Neisseria animaloris	0	1	0
Neisseria dumasiana	1	1	1
Pantoea agglomerans	0	1	0
Pasteurella canis	1	1	1
Staphylococcus pseudintermedius	3	2	1
Staphylococcus felis	0	0	1
Total	10	7	5

Table 7
Overview on MDR distribution in wounds.

Number of MDR	Distribution in wounds
0	59 % (30/51)
1	27 % (14/51)
> 1	14 % (7/51)

without MDR, $P = 0.003$). In six of these seven wounds (by two dogs) the adjustment in therapy consisted of adding marbofloxacin (2 mg/kg) based on susceptibility results. In one dog with five wounds, *Pseudomonas aeruginosa* resistant to amoxicillin-clavulanic acid was identified, along with *Escherichia coli* and a *Bacillus cereus* cluster that demonstrated intermediate susceptibility. The second dog, which had one wound with MDR, showed pathogens that were sensitive to amoxicillin-clavulanic acid.

The presence of MDR could not be linked to the development of minor or major complications or to a delayed wound closure. Specifically, complications occurred in 5/21 wounds with MDR compared to 3/30 wounds without MDR, and no complete wound closure on the tenth day after surgery was observed in 5/21 wounds with MDR compared to 2/30 wounds without MDR. Supplementary Table S2 shows the resistance profile of isolated MDR.

Antibiotics (AB)

Seven dogs with one wound each were randomly assigned to post-surgical treatment without prophylactic antibiotics. Minor complications were recorded in 3/7 wounds ($P = 0.015$) and major complications

in 2/7 wounds ($P = 0.033$) without initial antibiotic treatment, combined representing > 50 % of all complications ($P = 0.008$).

Three wounds without prophylactic AB (3/7) showed no complete wound closure at day ten, representing 43 % of all wounds without closure on day ten ($P = 0.045$).

Among these, 57 % (4/7) were categorized as two per Griffin and Holt (2001), and 43 % (3/7) were graded four (Griffin and Holt, 2001). Compared to other wounds in category two (n = 16), neither delayed wound closure nor complications were significantly more common in either group. In category four, complications could be associated with treatment without prophylactic antibiotic (3/3; $P = 0.010$). No difference in the occurrence of complications was observed between category four wounds that already received antibiotic pretreatment (n = 8) and those that received antibiotics intraoperatively (n = 13) (1/8 vs. 2/13; $P = 1.00$).

Discussion

The objective of this study was to compare the in vivo antiseptic efficacy of HOCl and PHMB in surgical treatment of DBW. Microbial wound contamination was evaluated, and a reduction in contamination scores during surgery was used to assess antiseptic efficacy. Wound healing was monitored, and potential factors influencing its progression were examined.

The present study found bacterial contamination rates in dog bite wounds (DBW) of 82 %; consistent with previous reports of 84– 96 % by Meyers et al. (2008), Mouro et al. (2010), Winter et al. (2018) and Kalnins et al. (2021). Lower contamination rates of 47 %- 68 % have been reported by other studies (Ateca et al., 2014; Frykfors von Hekkel et al.,2020; Griffin and Holt, 2001; Nolff et al., 2016; Nolff et al., 2019). The wide range of contamination rates between studies can be explained by the heterogeneous nature of bite wounds and differences in study design (Kalnins et al., 2021 Griffin and Holt, 2001). For example, Griffin and Holt (2001) excluded clinically infected wounds at presentation from their study, which likely explains a lower contamination rate. Studies including only severe DBW generally report higher contamination rates. In the present study, the relatively high contamination score can therefore be explained by the inclusion criteria of dogs with bite wounds, that required surgical treatment. In contrast, Ateca et al. (2014) found only 47 % positive cultures and Frykfors von Hekkel et al.,2020) reported 63 %, even though only severe bite wounds/ thoracic bite wounds were included in these retrospective studies. A possible explanation is that due to the retrospective nature of these studies not all included wounds were cultured. Furthermore, the timing of culturing (before or after debridement) varies between studies or is partially unknown due to their retrospective design.

With a wide variety of organisms isolated, the culture results of this study reaffirm the polymicrobial nature of dog bite wounds. Consistent with prior studies, the most common isolates included *Pasteurella* spp., *Staphylococcus* spp., *Streptococcus* spp. (Frykfors von Hekkel et al.,2020; Meyers et al., 2008; Nolff et al., 2016; Nolff et al., 2019; Winter et al., 2018), as well as *Neisseria* spp. (Kalnins et al., 2021; Nolff et al., 2019; Winter et al., 2018).

In two cases in the present study, a positive third culture followed a negative first culture. This phenomenon can potentially be explained by the design of the present study, the nature of managed wounds and semiquantitative testing. Bite wounds are contaminated with the victim's dermal microbiota, the attacker's oral microbiota and environmental bacteria (Bailey, 2011; Meyers et al., 2008). Bacteria in bite wounds are transported into the victim's deep tissue by the attacker's teeth (iceberg theory) (Holt and Griffin, 2000; Pavletic and Trout, 2006). Therefore, the pre-debridement microbiota may not always reflect the post-debridement microbiota in deeper tissues. In one other study examining sampling methods, a similar phenomenon was observed in one cultured bite wound, where a positive culture result after debridement and lavage followed a negative initial culture (Concannon et al., 2020). That veterinary study comparing microbiological swabbing and biopsy found no advantage of biopsy over swabbing confirming swab culture as a reliable method for clinical use (Concannon et al., 2020). Despite this, controversy remains for the best practice standard of testing. Wound biopsies are considered as the "gold standard" for microbiological testing in wounds in human medicine (Copeland-Halperin et al., 2016). However, as a reliable, non- invasive and inexpensive method, wound swabbing is most commonly used in clinical practice (Copeland-Halperin et al., 2016).

Previous studies that examined lavage efficacy in bite wound treatment and potential factors for the development of complications found no connection between any bacterial group and the development of complications (Nolff et al., 2019; Winter et al., 2018). The present study identified bacterial species that were more frequently associated with complications or impaired wound healing. *Streptococcus* species showed the strongest association in healing complications and cases of delayed wound closure. Similarly, *Neisseria* species were significantly linked to delayed wound closure and *Pasteurella* species were also identified in a majority of wounds with complications. This aligns with human studies that commonly identify *Streptococci* and *Pasteurella* species as the most common pathogens of infected DBWs (Abrahamian and Goldstein, 2011; Talan et al., 1999). *Neisseria* species are commonly found in canine oral microbiota (Bailie et al., 1978). Consistent with the present findings, *Neisseria* species have recently been linked to a non-healing DBW (Cobiella et al., 2019).

In the species- level analysis, *Staphylococcus pseudintermedius* could be linked to occurrence of complications in the present study. However, statistical support for its role in the present study was weaker. *Staphylococcus pseudintermedius* is commonly identified from healthy dogs as well as an opportunistic pathogen that could be associated with skin, ear and wound infections (Bannoehr and Guardabassi, 2012; Pompilio et al., 2015; Roberts et al., 2024).

Previous veterinary studies found *Staphylococcus pseudintermedius* (Kalnins et al., 2021; Meyers et al., 2008), *Streptococcus canis, Pasteurella canis, Pasteurella multocida* and *pyogenic staphylococci* (Meyers et al., 2008) more often in infected wound cultures compared to non- infected. However, the present data should be interpreted with caution, since wounds showed a polymicrobial environment and therefore, a distinct causality for infection cannot be concluded (Bowler et al., 2001). Nevertheless, these findings emphasizing the importance of microbiological testing for clinicians managing such wounds. Routine culturing of DBWs is recommended by multiple authors (Concannon et al., 2020; Kalnins et al., 2021) and is required in light of antimicrobial stewardship (BSAVA, 2024; Kalnins et al., 2021). In a previous study by Kalnins et al. (2021) only the largest wound of an individual was chosen for culture testing. The present study demonstrated that, within one individual with multiple wounds, culture results are not necessarily identical, and sampling each wound individually can provide important insights into differences in polymicrobial wound contamination and differences in resistance profiles. No other pre- surgical factors (time to presentation, wound location, number of wounds, pretreatment with antibiotic) or surgical parameters (use of drainage) were associated with wound healing complications. Identifying potential risk factors for DBW remains an important area for future investigations.

The rate of multidrug resistant bacteria (MDR) (41 %) in the present study is consistent with findings from Nolff et al. (2019) and Winter et al. (2018), which reported rates of 40 % and 20 % (38 % of first swab) MDR in positive cultures, respectively. These recently published values are higher than previously published data by Nolff et al. (2016), which reported MDR rates of 6 % in dog bite wounds. A recent retrospective study, that analyzed DBW data in Australia from 1999 to 2019 recorded a MDR rate of 35 % in bite wounds. Notably, in this study, only 2 % (27/1526) of all analyzed DBW cases were cultured, and the time of culture during the course of treatment remains unclear in many of the cases (Kalnins et al., 2022). In accordance with results from Winter et al. (2018), but contrary to results from Nolff et al. (2016), the isolation of

MDR in the present study did not always correlate with prior antibiotic treatment. However, the results from Nolff et al. (2016) must be interpreted with caution, as the sample size of bite wounds with MDR was considerably small (n = 5). In agreement with previous publications by Nolff et al. (2019) and Winter et al. (2018), no correlation was observed in the present study between the presence of MDR and complications in wound healing. The underlying reason for this finding remains unclear and should be explored in future studies.

The present data confirms that amoxicillin-clavulanic acid remains effective in many cases against the broad spectrum of bacteria found in DBWs. Consistent with this finding, a recent study by Kalnins et al. (2021) confirmed amoxicillin-clavulanic acid as the first choice for empirical treatment of DBWs. This recommendation aligns with previous reports (Kalnins et al., 2022; Meyers et al., 2008; Mouro et al., 2010) and with current antimicrobial usage guidelines by the British Small Animal Veterinary Association (BSAVA, 2024).

Nevertheless, the present data highlights the relevance of looking for alternatives beyond antibiotics. Nolff et al. (2019) demonstrated for both Polyhexanide (PHMB) ($P = 0.001$) and NaCl ($P = 0.037$) a highly significant decrease of wound bioburden after lavage. In direct intergroup comparison, PHMB showed an even better decontamination effect than NaCl after prelavage ($P = 0.006$) and main lavage ($P = 0.018$). Full wound decontamination in that study was achieved in 87 % (13/15) of PHMB- treated wounds, compared to 39 % (7/18) of NaCl – treated wounds. That study also highlighted the importance of an appropriate contact time of 15 min for PHMB and an increased antiseptic effect over time. Compared to its pilot study where the soak time had not been controlled, a complete decontamination was only achieved in 42 % of wounds treated with PHMB (Winter et al., 2018). In both the studies, NaCl lavage showed an overall good decontamination effect, achieving complete wound decontamination in 39 % (Nolff et al., 2019) and 50 % (Winter et al., 2018) of cases, respectively. Since PHMB demonstrated superior decontamination compared to NaCl in vivo after wound lavage, it was chosen as the control agent in the present study. Following the protocol of a controlled soak time, PHMB achieved complete decontamination in 84 % in the present study, consistent with previous data from Nolff et al. (2019).

In the present study, all tested agents (hypochlorous acid (HOCl) in two concentrations) showed similar decontamination efficiency to PHMB. Thus, the hypothesis that HOCl would achieve better decontamination than PHMB with a shorter soaking time had to be rejected. In this in vivo study, based on decontamination efficacy, no single agent achieved better results.

In absolute terms, PHMB demonstrated higher decontamination efficacy than both HOCl solutions, although this difference was not statistically significant.

In the context of clinical use, a shorter soaking time for HOCl compared to a minimum soaking time of 15 min for PHMB at similar decontamination efficacies seems advantageous. Furthermore, no significant difference in contamination efficiency was observed in the direct comparison between 0.015 % HOCl and 0.05 % HOCl. Nonetheless, in all cases, a good reduction in overall bioburden was achieved. This emphasizes the need for thorough debridement combined with physical irrigation. Both PHMB and HOCl are documented as highly effective antiseptics with almost immediate efficacy (Kramer et al., 2018; Sakarya et al., 2014), with the present study providing an in vivo validation for the immediate efficacy of HOCl, thereby marking an advantage over PHMB in clinical use.

In vitro studies have shown that both PHMB and HOCl are effective against microbial biofilms, reducing microbial bioburden while exhibiting no toxicity and promoting wound healing (Day et al., 2017; McMahon et al., 2020; Ortega-Peña et al., 2017; Rippon et al., 2023; Sakarya et al., 2014). A previous study by McMahon et al. (2020) using in vitro methods and an ex vivo model found varying biofilm reduction effects of PHMB and HOCl depending on the model used, which highlights the need for in vivo validation. The present study confirms their efficacy in microbial decontamination for wound treatment. In the available literature there are currently no other veterinary in vivo studies comparing the wound lavage efficacy of HOCl and most current recommendations are based on in vitro study results. Hence the present study provides urgently needed in vivo validation (Harriott et al., 2019) for these agents. Interestingly, one study found that HOCl performed better in liquid form than in gel (Ortega-Peña et al., 2017), likely due to the loss of the unstable active agents during or after the gel manufacturing process (Ortega-Peña et al., 2017). A stabilized HOCl liquid solution was used in the present study. Herruzo and Herruzo (2020) reported greater in vitro decontamination efficiency with higher-concentration HOCl in presence and absence of biofilm compared to lower- concentrated HOCl. This raises the question of why the same effect was not observed in the present in vivo study. One possible explanation is the relatively small concentration difference, which may not lead to a clinically observable distinction. Furthermore, the result of the present study should be interpreted with caution, as neither group showed significant results in direct comparison due to the relatively small sample size. Group imbalances in the current study were addressed statistically through the use of nonparametric analysis methods, such as the Kruskal-Wallis test, which are robust to unequal group sizes. In addition to unifactorial analysis, a multifactorial generalized linear model was applied, allowing the analysis to account for uneven distributions in wound classification grades. This approach ensured that observed differences were not merely due to sample size variation between treatment groups.

The hypothesis that surgical debridement with controlled lavage, without the use of prophylactic antibiotics, would suffice for treating bite wounds had to be rejected. Antibiotics are commonly used empirically in therapy of dog bite wounds (Kalnins et al., 2022). In human medicine, studies suggest using antibiotics only in high-risk wounds (Callaham, 1994). In veterinary medicine, Mouro et al. (2010) showed that less severe DBWs do not benefit from prophylactic antibiotic therapy and suggested administration in these wounds only in case of signs of infection. In the present study, the approach of withholding prophylactic antibiotics in the randomly allocated wounds and administering them only upon signs of infection occurred (Mouro et al., 2010), led to increased complications, requiring a change in study protocol. Re-operation was necessary in two cases due to major complications in form of necrosis, likely caused by circulatory disturbances in the wound area. Post -surgical skin necrosis can occur due to multiple factors, including infection and compromised blood supply in consequence of too much pressure after wound closure (Fossum, 2011). In both cases in the present study, necrosis could not be clinically associated with any major or minor sign of infection. In one wound *Neisseria dumasiana*, *Pasteurella canis*, and *Staphylococcus pseudintermedius* were detected in the third swab, while the other showed a negative third swab. None of the two wounds tested positive for *Fusobacterium necrophorum* at any of the sample points. However, a significant amount of skin was removed during initial debridement in both cases, which likely resulted in insufficient blood flow to the wound area after closure and became the most probable cause of necrosis.

In the present study a low overall complication rate (15 %) and a mortality rate of 0 % were found. In contrast, Ateca et al. (2014) reported a mortality rate of 15 % in their retrospective study. This difference to the present study may be explained by variations in inclusion criteria and patient data, as only severe injuries were included and 68 % of their patients weighed less than 10 kg. Frykfors von Hekkel et al., 2020) found a mortality rate of 15 %, associating the presence of pleural effusion with higher mortality. Notably, in the present study, no patients with pleural effusion were included. Nolff et al. (2019) reported a lower mortality rate of 5 %, with most fatal cases (n = 3/4) resulting from severe thoracic wall wounds.

Due to the low overall complication rate of 15 % (8/51 wounds) in the present study, data-based conclusions should be approached cautiously. Notably, 43 % (3/7) of wounds not showing closure by day

ten post-surgery were treated without prophylactic antibiotics. For low grade bite wounds (category two) in this study, neither delayed wound closure nor complications occurred significantly more often in either treatment group. However, for higher- grade bite wounds (category four), complications were significantly associated with the absence of prophylactic antibiotic treatment. Subsequently, the data suggests that dogs with higher- grade bite wounds would benefit from antibiotic treatment and that dogs with low-grade bite wounds requiring surgery may not need postoperative antimicrobial therapy if adequate debridement and antiseptic lavage are performed; as no difference in the healing process was observed regardless of antibiotic use. This aligns with recommendations by Mouro et al. (2010). It is important to note that the overall complication rate and number of wounds treated without any antibiotic was low; therefore, the present data can only provide an indication and highlight the need for further investigations. Thus, an unrestricted recommendation cannot be made.

In category four wounds, complication rates did not differ between those receiving antibiotic premedication and those starting antibiotic treatment intraoperatively. These findings indicate that the timing of antibiotic administration may be less critical than the quality of surgical wound management. As the overall complication numbers were low and the exact timing of premedication was not always available, these findings should be interpreted with caution. Further studies with larger sample sizes are needed to confirm these observations.

The follow-up period in this study concluded with suture removal. Paeckel et al. (2024) reported that surgical site infections can occur up to 30 days after suture removal. In that retrospective study, 7 % of patients undergoing clean surgeries developed surgical site infections, 85 % of which occurred before suture removal (Paeckel et al., 2024). Accordingly, the possibility of undocumented postoperative complications in the present study cannot be entirely excluded, representing a limitation of the current study.

The data from the present study demonstrated that non- severe DBW in otherwise healthy dogs, when treated appropriately with an antiseptic agent, did not require further systemic antimicrobial treatment. This aligns with findings from previous studies on both DBW and dog bites in humans (Callaham, 1994; Cummings, 1994; Mouro et al., 2010; Smith et al., 2000) and supports recent recommendations from a review by Kalnins et al. (2022) to avoid the use of antimicrobials in low-grade DBW.

Conclusions

Identified bacteria were in accordance those found in previous studies. However, the proportion of MDR isolates was higher and certain bacteria groups were cultured significantly more often in wounds with delayed wound healing. No superior antiseptic agent for wound lavage was identified among those tested. Both antiseptic agents showed very good wound decontamination in vivo. In direct comparison, the almost immediate effect and shorter soaking time of HOCl- based antiseptics represent a practical advantage for clinical use. Despite good contamination effects of antiseptic lavages, antibiotic prophylactics should still be considered in dogs with severe bite wounds. There are indications that prophylactic antibiotics may not be needed in low grade bite wounds. This gives an important angle for further investigations especially in regard of antibiotic stewardship.

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CRediT authorship contribution statement

M. Peters: Methodology, Investigation, Data curation, Conceptualization, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **D. Eberle:** Investigation, Conceptualization, Validation, Supervision, Writing – review & editing. **S. Reese:** Methodology, Formal analysis, Visualization, Validation, Writing – review & editing. **G. Wolf:** Investigation, Validation, Writing – review & editing. **A. Meyer- Lindenberg:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tvjl.2025.106505.

References

Abbas, A., Barkhouse, A., Hackenberger, D., Wright, G.D., 2024. Antibiotic resistance: a key microbial survival mechanism that threatens public health. Cell Host & Microbe 32, 837–851. https://doi.org/10.1016/j.chom.2024.05.015.

Abrahamian, F.M., Goldstein, E.J., 2011. Microbiology of animal bite wound infections. Clinical Microbiology Reviews 24, 231–246. https://doi.org/10.1128/CMR.00041-10.

Andrés, C.M.C., Pérez de la Lastra, J.M., Juan, C.A., Plou, F.J., Pérez-Lebeña, E., 2022. Hypochlorous acid chemistry in mammalian cells-influence on infection and role in various pathologies. International Journal of Molecular Sciences 23. https://doi.org/10.3390/ijms231810735.

Aragón-Sánchez, J., Quintana-Marrero, Y., Aragón-Hernández, C., Hernández-Herero, M. J., 2017. ImageJ: a free, easy, and reliable method to measure leg ulcers using digital pictures. The International Journal of Lower Extremity Wounds 269–273. https://doi.org/10.1177/1534734617744951.

Ateca, L.B., Drobatz, K.J., King, L.G., 2014. Organ dysfunction and mortality risk factors in severe canine bite wound trauma. Journal of Veterinary Emergency and Critical Care 24, 705–714. https://doi.org/10.1111/vec.12256.

Bailey, D., 2011. Forensic veterinary science of bite wounds. The Veterinary Record 168, 650. https://doi.org/10.1136/vr.d3569.

Bailie, W.E., Stowe, E.C., Schmitt, A.M., 1978. Aerobic bacterial flora of oral and nasal fluids of canines with reference to bacteria associated with bites. Journal of Clinical Microbiology 7, 223–231. https://doi.org/10.1128/jcm.7.2.223-231.1978.

Bannoehr, J., Guardabassi, L., 2012. Staphylococcus pseudintermedius in the dog: taxonomy, diagnostics, ecology, epidemiology and pathogenicity. e251-252 Veterinary Dermatology 23, 253–266. https://doi.org/10.1111/j.1365-3164.2012.01046.x.

Banovic, F., Bozic, F., Lemo, N., 2013. In vitro comparison of the effectiveness of polihexanide and chlorhexidine against canine isolates of Staphylococcus pseudintermedius, Pseudomonas aeruginosa and Malassezia pachydermatis. e488-409 Veterinary Dermatology 24, 409–413. https://doi.org/10.1111/vde.12048.

Barreto, R., Barrois, B., Lambert, J., Malhotra-Kumar, S., Santos-Fernandes, V., Monstrey, S., 2020. Addressing the challenges in antisepsis: focus on povidone iodine. International Journal of Antimicrobial Agents 56, 106064. https://doi.org/10.1016/j.ijantimicag.2020.106064.

Bonham, P.A., 2009. Swab cultures for diagnosing wound infections: a literature review and clinical guideline. Journal of Wound Ostomy and Continence Nursing Official Publication of the Wound Ostomy and Continence Nurses Society / WOCN 36, 389–395. https://doi.org/10.1097/WON.0b013e3181aaef7f.

Bowler, P.G., Duerden, B.I., Armstrong, D.G., 2001. Wound microbiology and associated approaches to wound management. Clinical Microbiology Reviews 14, 244–269. https://doi.org/10.1128/cmr.14.2.244-269.2001.

BSAVA, 2024. Antibacterial use in our practice. Guide to Responsible Use of Antibacterials: PROTECT ME., 2024. BSAVA, Gloucester. ⟨https://www.bsavalibrary.com/content/chapter/10.22233/9781913859312.app11#html_fulltext⟩ (accessed January 2025).

Callaham, M., 1994. Prophylactic antibiotics in dog bite wounds: nipping at the heels of progress. Annals of Emergency Medicine 23, 577–579. https://doi.org/10.1016/s0196-0644(94)80284-x.

Chang, A.C., Dearman, B., Greenwood, J.E., 2011. A comparison of wound area measurement techniques: visitrak versus photography. Eplasty 11, e18.

CLSI, 2024. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals, 6th ed. Clinical and Laboratory Standards Institute.

Cobiella, D., Gram, D., Santoro, D., 2019. Isolation of Neisseria dumasiana from a deep bite wound infection in a dog. Veterinary Dermatology 30, 556–e168. https://doi.org/10.1111/vde.12791.
Concannon, T.M., Kidd, L., Osmond, C., Dong, F., Griffon, D., 2020. Prospective evaluation of the influence of sampling method on bacterial culture results and antimicrobial selection in 52 dogs with infected wounds. Journal of Veterinary Emergency and Critical Care 30, 149–158. https://doi.org/10.1111/vec.12926.
Copeland-Halperin, L.R., Kaminsky, A.J., Bluefeld, N., Miraliakbari, R., 2016. Sample procurement for cultures of infected wounds: a systematic review, 6, s8-S10 Journal of Wound Care 25, S4. https://doi.org/10.12968/jowc.2016.25.Sup4.S4.
Cummings, P., 1994. Antibiotics to prevent infection in patients with dog bite wounds: a meta-analysis of randomized trials. Annals of Emergency Medicine 23, 535–540. https://doi.org/10.1016/s0196-0644(94)70073-7.
da Cruz Nizer, W.S., Inkovskiy, V., Overhage, J., 2020. Surviving reactive chlorine stress: responses of gram-negative bacteria to hypochlorous acid. Microorganisms 8. https://doi.org/10.3390/microorganisms8081220.
Daeschlein, G., 2013. Antimicrobial and antiseptic strategies in wound management. International Wound Journal 10 (1), 9–14. https://doi.org/10.1111/iwj.12175.
Day, A., Alkhalil, A., Carney, B.C., Hoffman, H.N., Moffatt, L.T., Shupp, J.W., 2017. Disruption of biofilms and neutralization of bacteria using hypochlorous acid solution: an in vivo and in vitro evaluation. Advances in Skinner Wound Care 30, 543–551. https://doi.org/10.1097/01.Asw.0000526607.80113.66.
Dernell, W.S., 2006. Initial wound management. The Veterinary Clinics of North America Small Animal Practice 36, 713–738. https://doi.org/10.1016/j.cvsm.2006.04.003.
Eberlein, T., Assadian, O., 2010. Clinical use of polihexanide on acute and chronic wounds for antisepsis and decontamination. Skinner Pharmacology and Physiology 23, 45–51. https://doi.org/10.1159/000318267.
Fossum, T.W., 2011. Chirurgie der Kleintiere, second ed. Elsevier Health Sciences, Germany.
Frykfors von Hekkel, A.K., Pegram, C., Halfacree, Z.J., 2020. Thoracic dog bite wounds in dogs: A retrospective study of 123 cases (2003-2016). Veterinary Surgery VS the Official Journal of the American College of Veterinary Surgeons 49, 694–703. https://doi.org/10.1111/vsu.13402.
Gandolfi-Decristophoris, P., Regula, G., Petrini, O., Zinsstag, J., Schelling, E., 2013. Prevalence and risk factors for carriage of multi-drug resistant Staphylococci in healthy cats and dogs. Journal of Veterinary Science 14, 449–456. https://doi.org/10.4142/jvs.2013.14.4.449.
Goertz, O., Lauer, H., Hirsch, T., Daigeler, A., Harati, K., Stricker, I., Lehnhardt, M., von der Lohe, L., 2016. Evaluation of angiogenesis, epithelialisation and microcirculation after application of polyhexanide, chitosan and sodium chloride in rodents. International Wound Journal 13, 1161–1167. https://doi.org/10.1111/iwj.12434.
Gold, M.H., Andriessen, A., Bhatia, A.C., Bitter Jr., P., Chilukuri, S., Cohen, J.L., Robb, C. W., 2020. Topical stabilized hypochlorous acid: the future gold standard for wound care and scar management in dermatologic and plastic surgery procedures. Journal of Cosmetic Dermatology 19, 270–277. https://doi.org/10.1111/jocd.13280.
Griffin, G.M., Holt, D.E., 2001. Dog-bite wounds: bacteriology and treatment outcome in 37 cases. Journal of the American Animal Hospital Association 37, 453–460. https://doi.org/10.5326/15473317-37-5-453.
Harriott, M.M., Bhindi, N., Kassis, S., Summitt, B., Perdikis, G., Wormer, B.A., Rankin, T. M., Kaoutzanis, C., Samaha, M., Stratton, C., Schmitz, J.E., 2019. Comparative Antimicrobial Activity of Commercial Wound Care Solutions on Bacterial and Fungal Biofilms. Annals of Plastic Surgery 83, 404–410. https://doi.org/10.1097/sap.0000000000001996.
Herruzo, R., Herruzo, I., 2020. Antimicrobial efficacy of a very stable hypochlorous acid formula compared with other antiseptics used in treating wounds: in-vitro study on micro-organisms with or without biofilm. The Journal of Hospital Infection 105, 289–294. https://doi.org/10.1016/j.jhin.2020.01.013.
Holt, D.E., Griffin, G., 2000. Bite wounds in dogs and cats (viii). The Veterinary Clinics of North America Small Animal Practice 30, 669–679. https://doi.org/10.1016/s0195-5616(00)50045-x.
Hübner, N.O., Kramer, A., 2010. Review on the efficacy, safety and clinical applications of polihexanide, a modern wound antiseptic. Skinner Pharmacology and Physiology 23, 17–27. https://doi.org/10.1159/000318264.
Kaehn, K., 2010. Polihexanide: a safe and highly effective biocide (Suppl). Skinner Pharmacology and Physiology 23, 7–16. https://doi.org/10.1159/000318237.
Kalnins, N.J., Gibson, J.S., Stewart, A.J., Croton, C., Purcell, S.L., Rajapaksha, B., Haworth, M., 2022. Antimicrobials in dog-to-dog bite wounds: a retrospective study of 1526 dog bite events (1999-2019). Journal of Veterinary Internal Medicine American College of Veterinary Internal Medicine 36, 2028–2041. https://doi.org/10.1111/jvim.16574.
Kalnins, N.J., Haworth, M., Croton, C., Gibson, J.S., Stewart, A.J., Purcell, S.L., 2021. Treatment of moderate grade dog bite wounds using amoxicillin-clavulanic acid with and without enrofloxacin: a randomised non-inferiority trial. Australian Veterinary Journal 99, 369–377. https://doi.org/10.1111/avj.13093.
Koburger, T., Hübner, N.O., Braun, M., Siebert, J., Kramer, A., 2010. Standardized comparison of antiseptic efficacy of triclosan, PVP-iodine, octenidine dihydrochloride, polyhexanide and chlorhexidine digluconate. The Journal of Antimicrobial Chemotherapy 65, 1712–1719. https://doi.org/10.1093/jac/dkq212.
Kramer, A., Dissemond, J., Kim, S., Willy, C., Mayer, D., Papke, R., Tuchmann, F., Assadian, O., 2018. Consensus on Wound Antisepsis: Update 2018. Skinner Pharmacology and Physiology 31, 28–58. https://doi.org/10.1159/000481545.
Kramer, A., Eberlein, T., Muller, G., Dissemond, J., Assadian, O., 2019. Re-evaluation of polihexanide use in wound antisepsis in order to clarify ambiguities of two animal studies. Journal of Wound Care 28, 246–255. https://doi.org/10.12968/jowc.2019.28.4.246.
Lee, W.R., Tobias, K.M., Bemis, D.A., Rohrbach, B.W., 2004. In vitro efficacy of a polyhexamethylene biguanide-impregnated gauze dressing against bacteria found in veterinary patients. Veterinary Surgery VS the Official Journal of the American College of Veterinary Surgeons 33, 404–411. https://doi.org/10.1111/j.1532-950X.2004.04059.x.
Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D.L., Rice, L.B., Stelling, J., Struelens, M.J., Vatopoulos, A., Weber, J.T., Monnet, D.L., 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection the Official Publication of the European Society of Clinical Microbiology and Infectious Diseases 18, 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x.
McCaghertyx, J., Woods, S., 2018. Wound management part one: topical treatments. Companion Animal 23, 696–701. https://doi.org/10.12968/coan.2018.23.12.696.
McMahon, R.E., Salamone, A.B., Poleon, S., Bionda, N., Salamone, J.C., 2020. Efficacy of Wound Cleansers on Wound-Specific Organisms Using In Vitro and Ex Vivo Biofilm Models. Wound Manag Prev 66, 31–42.
Medina-Tamayo, J., Sanchez-Miranda, E., Balleza-Tapia, H., Ambriz, X., Cid, M.E., Gonzalez-Espinosa, D., Gutierrez, A.A., Gonzalez-Espinosa, C., 2007. Super-oxidized solution inhibits IgE-antigen-induced degranulation and cytokine release in mast cells. International Immunopharmacology 7, 1013–1024. https://doi.org/10.1016/j.intimp.2007.03.005.
Meyers, B., Schoeman, J.P., Goddard, A., Picard, J., 2008. The bacteriology and antimicrobial susceptibility of infected and non-infected dog bite wounds: fifty cases. Veterinary Microbiology 127, 360–368. https://doi.org/10.1016/j.vetmic.2007.09.004.
Mouro, S., Vilela, C.L., Niza, M.M., 2010. Clinical and bacteriological assessment of dog-to-dog bite wounds. Veterinary Microbiology 144, 127–132. https://doi.org/10.1016/j.vetmic.2009.12.042.
Müller, G., Kramer, A., 2008. Biocompatibility index of antiseptic agents by parallel assessment of antimicrobial activity and cellular cytotoxicity. The Journal of Antimicrobial Chemotherapy 61, 1281–1287. https://doi.org/10.1093/jac/dkn125.
Nolff, M.C., Reese, S., Fehr, M., Dening, R., Meyer-Lindenberg, A., 2016. Assessment of wound bio-burden and prevalence of multi-drug resistant bacteria during open wound management. The Journal of Small Animal Practice 57, 255–259. https://doi.org/10.1111/jsap.12476.
Nolff, M.C., Winter, S., Reese, S., Meyer-Lindenberg, A., 2019. Comparison of polyhexanide, cold atmospheric plasma and saline in the treatment of canine bite wounds. The Journal of Small Animal Practice 60, 348–355. https://doi.org/10.1111/jsap.12971.
Ortega-Peña, S., Hidalgo-González, C., Robson, M.C., Krötzsch, E., 2017. In vitro microbicidal, anti-biofilm and cytotoxic effects of different commercial antiseptics. International Wound Journal 14, 470–479. https://doi.org/10.1111/iwj.12625.
Paeckel, N., Zablotski, Y., Meyer-Lindenberg, A., 2024. The effect of peri- and postoperative antibiotic prophylaxis on surgical site infection in surgeries with elective antibiotic administration. Veterinary Journal (London England 1997) 308, 106267. https://doi.org/10.1016/j.tvjl.2024.106267.
Pavletic, M.M., Trout, N.J., 2006. Bullet, bite, and burn wounds in dogs and cats. The Veterinary Clinics of North America Small Animal Practice 36, 873–893. https://doi.org/10.1016/j.cvsm.2006.02.005.
Pompilio, A., De Nicola, S., Crocetta, V., Guarnieri, S., Savini, V., Carretto, E., Di Bonaventura, G., 2015. New insights in Staphylococcus pseudintermedius pathogenicity: antibiotic-resistant biofilm formation by a human wound-associated strain. BMC Microbiology 15, 109. https://doi.org/10.1186/s12866-015-0449-x.
QuickCalc, GraphPad, 2021. ⟨http://www.graphpad.com/quickcalcs/ConfInterval1.cfm⟩ (accessed August 2021).
Rippon, M., Rogers, A.A., Westgate, S., Ousey, K., 2023. Effectiveness of a polyhexamethylene biguanide-containing wound cleansing solution using experimental biofilm models. Journal of Wound Care 32, 359–367. https://doi.org/10.12968/jowc.2023.32.6.359.
Roberts, E., Nuttall, T.J., Gkekas, G., Mellanby, R.J., Fitzgerald, J.R., Paterson, G.K., 2024. Not just in man's best friend: a review of Staphylococcus pseudintermedius host range and human zoonosis. Research in Veterinary Science 174, 105305. https://doi.org/10.1016/j.rvsc.2024.105305.
Sakarya, S., Gunay, N., Karakulak, M., Ozturk, B., Ertugrul, B., 2014. Hypochlorous Acid: an ideal wound care agent with powerful microbicidal, antibiofilm, and wound healing potency. Wounds 26, 342–350.
Smith, P.F., Meadowcroft, A.M., May, D.B., 2000. Treating mammalian bite wounds. Journal of Clinical Pharmacy and Therapeutics 25, 85–99. https://doi.org/10.1046/j.1365-2710.2000.00274.x.
Smith, M.R., Walker, A., Brenchley, J., 2003. Barking up the wrong tree? A survey of dog bite wound management. Emergency Medicine Journal EMJ 20, 253–255. https://doi.org/10.1136/emj.20.3.253.
Talan, D.A., Citron, D.M., Abrahamian, F.M., Moran, G.J., Goldstein, E.J., 1999. Bacteriologic analysis of infected dog and cat bites. Emergency Medicine Animal Bite Infection Study Group N Engl J Med 340, 85–92. https://doi.org/10.1056/NEJM199901143400202.
Wound Infection in Clinical Practice. Int Wound J 5 (Suppl 3), 2008, iii–11. https://doi.org/10.1111/j.1742-481X.2008.00488.x.
Uri, M., Buckley, L.M., Marriage, L., McEwan, N., Schmidt, V.M., 2016. A pilot study comparing in vitro efficacy of topical preparations against veterinary pathogens. Veterinary Dermatology 27, 152–e139. https://doi.org/10.1111/vde.12306.
Wang, L., Bassiri, M., Najafi, R., Najafi, K., Yang, J., Khosrovi, B., Hwong, W., Barati, E., Belisle, B., Celeri, C., Robson, M.C., 2007. Hypochlorous acid as a potential wound

care agent: part I. Stabilized hypochlorous acid: a component of the inorganic armamentarium of innate immunity. Journal of Burns and Wounds 6, e5.
Winter, M., Boecker, D., Posch, W., 2025. Hypochlorous acid (HOCl) as a promising respiratory antiseptic. Viruses 17. https://doi.org/10.3390/v17091219.
Winter, S., Nolff, M.C., Reese, S., Meyer-Lindenberg, A., 2018. Comparison of the antibacterial efficacy of polyhexanide, cold atmospheric argon plasma and saline in the treatment of canine bite wounds. Tierarztlich Praxis Ausgabe K Kleintiere Heimtiere 46, 73–82. https://doi.org/10.15654/TPK-170713.