Berl Münch Tierärztl Wochenschr 131, 481–486 (2019)
DOI 10.2376/0005-9366-18044

ISSN 0005-9366

Korrespondenzadresse:
dvnuk@vef.hr

Eingegangen: 04.05.2018
Angenommen: 03.12.2018

Online first: 03.07.2019
http://vetline.de/facharchiv/158/3222

Faculty of Veterinary Medicine, Prilepska n.n., Bitola, R. Macedonia[1]
Croatian Veterinary Institute, Zagreb, Croatia[2]
Clinic of Surgery, Orthopaedics and Ophthalmology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia[3]

Presurgical antiseptic efficacy of chlorhexidine gluconate and povidone-iodine on the mucous membrane of mandibular gingiva in dogs

Präoperative antiseptische Wirksamkeit von Chlorhexidingluconat und Povidon-Jod auf der mandibulären gingivalen Schleimhaut bei Hunden

Panče Dameski[1], Boris Habrun[2], Mirta Vučković[3], Dražen Matičić[3], Andrija Musulin[3], Gordan Kompes[2], Dražen Vnuk[3]

Summary

Since in dogs the bacterial count is higher in the oral cavity than on the skin surface, it is very important to select an effective antiseptic that would greatly reduce the number of intraoral pathogenic microorganisms, thereby reducing the frequency of postoperative complications, especially postoperative infections after intraoral procedures.
The objective of this study was to investigate the basic aerobic bacterial microflora in the dog mouth and to investigate the antiseptic efficacy of 0.4% chlorhexidine gluconate and 1% povidone-iodine on mandibular gingival mucous membrane. A total of 45 dogs were divided into three groups. The CH group was treated with 0.4% chlorhexidine gluconate, the PI group with 1% povidone-iodine and the SL group with saline. Swabs were taken from the mandibular gingiva, before and after treatment with antiseptic solution. The number of bacteria was determined using the semiquantitative method, and the identification of bacterial colonies was performed after colonization of individual colonies on blood agar. Of the total 90 swabs, 30 species of aerobic bacteria were isolated and identified. Regarding antiseptic efficiency, both chlorhexidine gluconate and povidone-iodine showed statistically significant reductions of growth of bacterial colonies compared to the control group.
There were no differences between the two tested antiseptics regarding their efficacy in reducing the growth of bacterial colonies, and the number of positive swabs obtained after rinsing with chlorhexidine gluconate and povidone-iodine was identical. Based on the results, a 2-minute flush with 0.4% chlorhexidine gluconate or 1% povidone-iodine is recommended for presurgical preparation of the oral cavity in dogs.

Keywords: microorganism, reduction, canine oral cavity, flushing

Zusammenfassung

Aufgrund der Tatsache, dass die Zahl der Bakterien im Maul von Hunden größer ist als die Zahl der Bakterien auf der Hautoberfläche, ist es sehr wichtig, ein Antiseptikum zu wählen, das wirksam die Zahl von intraoralen pathogenen Mikroorganismen reduziert und damit die Häufigkeit postoperativer Komplikationen vermindert, insbesondere die Zahl der postoperativen Infektionen nach intraoralen Prozeduren.
Das Ziel dieser Studie war, die grundsätzliche aerobe Bakterienmikroflora im Maul des Hundes zu untersuchen und die antiseptische Wirksamkeit von 0,4 % Chlorhexidingluconat- und 1 % Povidon-Jod-Lösung auf der mandibulären gingivalen Schleimhaut zu ermitteln. Insgesamt wurden 45 Hunde untersucht, die in drei Gruppen eingeteilt waren. Die CH-Gruppe wurde mit 0.4 % Chlorhexidingluconat-Zubereitung behandelt, die PI-Gruppe mit 1 % Povidon-Jod-Lösung und die SL-Gruppe mit Kochsalzlösung. Abstriche wurden vor und nach der Behandlung mit der jeweiligen Lösung von der mandibulären Gingiva genommen. Die Zahl der Bakterien wurde mit einer semiquatitativen Methode bestimmt und die Identifikation der Bakterienkolonien erfolgte nach dem Wachstum individueller

Kolonien auf den Blutagars. Von den insgesamt 90 genommenen Abstrichen konnten 30 Spezies aerober Bakterien isoliert und identifiziert werden. In Bezug auf die antiseptische Wirksamkeit zeigten sowohl Chlorhexidingluconat als auch Povidon-Jod eine statistisch signifikante Verringerung des Wachstums von Bakterienkolonien im Vergleich mit der Kontrollgruppe.
Es gab keinen Unterschied zwischen diesen beiden Antiseptika, sowohl im Hinblick auf die Anzahl an Bakterienkolonien als auch auf die Zahl der positiven Abstriche nach Behandlung mit Chlorhexidingluconat beziehungsweise Providon-Jod. Aufgrund der Ergebnisse dieser Studie wird eine zwei Minuten dauernde Spülung mit 0,4 % Chlorhexidingluconat oder mit 1 % Povidon-Jod für die präoperative Vorbereitung des Mauls beim Hund empfohlen.

Schlüsselwörter: Antiseptikum, Gingiva, Spülung, Maulhöhle

Introduction

Surgical procedures in the oral cavity are often performed in veterinary clinics, mostly in dogs. The most common tumours of the oral cavity are epulides, planocellular carcinomas, fibrosarcomas, malignant melanomas and osteosarcoma (Ramos-Vara et al. 2000). The most common therapeutic protocol for oral neoplasms includes surgical treatment. Since certain procedures, such as tooth extraction, or certain pathological processes, such as hyperplasia of the gingiva, require a surgical approach, proper preparation of the surgical site in the oral cavity is of great importance.

Surgical site infections (SSI) are among the more significant complications associated with surgical treatment. In veterinary medicine, SSI is described as a complication that occurs in 0.8 to 18.1% cases in small animal surgery, with significant variations depending on the type of surgical procedure (Beal et al. 2000, Eugster et al. 2004,Vasseur et al. 1988, Weese 2008, Weese and Halling 2006, Whittem et al. 1999).

To improve surgical techniques and to prevent surgical site infections (SSI), it is important to respect the principals of asepsis and antisepsis. This increases the success rate of surgical procedure, and reduces complications (especially infections) to a minimum.

In this study, the bacteria isolated from the mouths of dogs were similar to those found on the skin surface and in the canine preputial cavity. These bacteria include potentially pathogenic bacteria, such as *Pasteurella (P.) multocida*, β-haemolytic *Streptococcus* sp., *Escherichia (E.) coli*, *Staphylococcus (S.) aureus* (Bjurstrom and Linde-Forsberg 1992, Neihaus et al. 2011, Scott et al. 2001). Due to this rich bacterial population, effective presurgical antisepsis of the oral cavity is essential.

In veterinary medicine, few studies have addressed the antiseptic efficacy of chlorhexidine gluconate and povidone-iodine in the oral cavity of dogs. Therefore, one of the aims of this study was to scientifically determine the antiseptic efficacy of chlorhexidine gluconate and povidone-iodine on the gingiva of dogs, the degree of reduction of pathogenic bacteria after using these antiseptics, and the differences between the test groups depending on the antiseptic used.

Materials and Methods

Animals

The dogs presenting for various surgical procedures were sampled. A total of 45 dogs met the criteria and were included in study. Observing the complete population of dogs (n=45), the most common breeds were mixed-breeds (n=24), Macedonian shepherd dog (n=4), Siberian husky (n=2), and French bulldog (n=2). Other breeds included in the study were Pug, Pekingese, Bullmastiff, Croatian sheepdog, Bulgarian shepherd dog, Epagneul Breton, Samoyed, Cane corso, Weimaraner dog, Labrador retriever, English Bulldog, Great dane and miniature pinscher (n=1). The mean age of dogs at the time of sampling was 3,16±2,35 years (range 1–13 years). Eighteen female (ten intact and eight spayed) and twenty seven male (twenty intact, seven neutered) were included in study. The mean body weight at the time of sampling was 20,73±11,18 kg (range 4–53 kg).

Dogs were excluded if they were less than one year of age, had visible pathological processes in the mouth, or had received antibiotics for any reason in the previous 14 days.

Procedure

Dogs were randomly divided into three groups of fifteen dogs using computer programmed randomisation. One group (PI) was treated with 1% povidone-iodine (Betadine 10%, Alkaloid, Skopje, F.Y.R.Macedonia), one group (CH) was treated with 0.4% chlorhexidine gluconate (PLIVA® sept glukonat, Pliva, Zagreb, Croatia), and the control group (SL) was treated with 0.9% saline (Natrii chloridum, B. Braun 9 mg/ml, Melsungen, Germany). Antiseptic solutions were prepared daily. The CH solution was prepared by adding 5% chlorhexidine gluconate solution to sterile water for injection. The PI solution was prepared by adding 10% povidone-iodine solution to 0.9% saline, and the original 0.9% saline solution was used as the control. After premedication and induction of anaesthesia, all dogs were intubated and placed in sternal recumbency.

Commercial kits with transport media was used for taking swabs. Swabs were taken at the level of the mandibular third premolar. A commercial swab was premoistened with 2–3 drops sterile saline solution. The mucous membrane of the buccal mandibular gingiva (2 x 2 cm area close to fourth premolar and first molar) was flushed twice using 10 ml sterile saline. After flushing, samples were taken by rotating swabs on the mucous membrane for 5 seconds to ensure adequate sampling. Further, the mucous membrane of the buccal mandibular gingiva was flushed six times with 10 ml antiseptic for 2 minutes for the CH and PI group, and with saline for the control group. After flushing, the gingiva of all dogs was flushed twice with 10 ml 0.9% sterile saline solution to remove residual antiseptic before col-

TABLE 1: *Overview of aerobic bacteria identified from mandibular gingiva prior to and following antiseptic flushing with 1% povidone-iodine (PI). Data are presented as number of positive swabs of total 15 (percent).*

Bacteria	Pre-flush PI	Post-flush PI
Pasteurella multocida	5/15 (33.3 %)	1/15 (6.6 %)
Escherichia coli	5/15 (33.3 %)	0
Staphylococcus aureus	4/15 (26.6 %)	0
Enterococcus sp.	3/15 (20 %)	1/15 (6.6 %)
Neisseria sp.	3/15 (20 %)	0
Bergeyella zoohelcum	2/15 (13.3 %)	0
Streptococcus sp.	2/15 (13.3 %)	0
β-hemolytic *Streptococcus* sp.	1/15 (6.6 %)	1/15 (6.6 %)
Enterococcus faecalis	1/15 (6.6 %)	1/15 (6.6 %)
Staphylococcus sciuri	1/15 (6.6 %)	0
Staphylococcus lentus	1/15 (6.6 %)	0
Moraxella sp.	1/15 (6.6 %)	0
Corynebacterium sp.	1/15 (6.6 %)	0
Stomatococcus mucilaginosus	1/15 (6.6 %)	0
Miscellaneuous Gram Negative Bacilli	1/15 (6.6 %)	0
Corynebacterium renale	1/15 (6.6 %)	0
Klebsiella oxyctoca	1/15 (6.6 %)	0
Pasteurella canis	1/15 (6.6 %)	0
Streptococcus pyogenes	1/15 (6.6 %)	0
Erysipelotrix rhusiopathie	1/15 (6.6 %)	0

TABLE 2: *Overview of aerobic bacteria identified from the mandibular gingiva prior to and following antiseptic flushing with 0.4% chlorhexidine gluconate (CH). Data are presented as number of positive swabs of total 15 (percent).*

Bacteria	Pre-flush CH	Post-flush CH
Escherichia coli	7/15 (46.6 %)	0
Staphylococcus aureus	5/15 (33.3 %)	0
Enterococcus sp.	5/15 (33.3 %)	3/15 (20 %)
Neisseria sp.	4/15 (26.6 %)	0
β-hemolytic *Streptococcus* sp.	3/15 (20 %)	1/15 (6.6 %)
Pasteurella multocida	2/15 (13.3 %)	0
Pasteurella canis	2/15 (13.3 %)	0
Bergeyella zoohelcum	2/15 (13.3 %)	0
Enterococcus faecium	2/15 (13.3 %)	0
Micrococcus sp.	2/15 (13.3 %)	0
Streptococcus sp.	2/15 (13.3 %)	0
Klebsiella sp.	1/15 (6.6 %)	1/15 (6.6 %)
Leuconostoc sp.	1/15 (6.6 %)	1/15 (6.6 %)
Bacillus cereus	1/15 (6.6 %)	0
Corinebacterium sp.	1/15 (6.6 %)	0
Moraxella sp.	1/15 (6.6 %)	0
Proteus sp.	1/15 (6.6 %)	0

lecting the post-flushing swab for culture using the same technique as described for the antiseptic.

All samples were collected by the same person (PD). Sterile gloves were worn for all sample collections. Five minutes after sample collection, the mucosa of the gingiva was examined for evidence of tissue reaction, such as erythema or wheals. No dogs in any of the groups displayed any adverse reactions after flushing.

After taking the swab from the mucosa, the swabs were refrigerated at 4°C until they were transferred to a reference diagnostic laboratory. All samples were plated within 24 h of collection. All isolations and culture evaluations were performed by the same microbiologist who was blinded to the type of flush solution tested. Swabs were plated on:

1. Blood agar, containing 5% sterile defibrinated ovine blood. This agar was used for the separation and cultivation of various fastidious bacteria, especially pathogenic species, as used for determination of haemolysis.
2. Nutritious agar (NA). This agar is an appropriate medium for cultivation of less-fastidious bacterial species. The nutritious substances in the agar from peptone, flesh extract and vitamins from yeast extract are favourable for the growth of most bacteria.
3. Xylose-Lysine-Desoxycholate agar (XLD). This is a selective agar for isolating bacteria of the *Enterobacteriae* family.

All agars were incubated at 37°C in ambient air. Cultures were examined after 24 h and 48 h of incubation. Semiquantitative scoring of total bacterial growth was performed using the quadrant streak method. Growth was recorded as none (0), very light for ≤10 colonies in the first quadrant only (1), light for >10 colonies in the first quadrant only (2), moderate for growth spreading into the second quadrant (3) and heavy for growth that spread into the third or fourth quadrant (4).

Statistical analysis

Data were analysed using statistical software Stata 13.1 (Stata corp. USA). The degree of difference on the gingiva was coded from 0 to 4. The correlation between the degree of difference of the gingiva after treatment was determined using univariant statistical analysis with χ^2 analysis and Fisher's exact test (Tab. 4). Asymptotic Generalized Pearson Chi-Squared Test for ordinal variables was used to test the association between the treatment groups and bacterial growth scores with, if significant, a post-hoc ordinal independence test for pairwise comparison of treatment groups (Tab. 5 and Tab. 6). Statistical significance was set at $p<0.05$.

Results

In the PI group, the bacterial species isolated after rinsing were: β-haemolytic *Streptococcus* sp., *P. multocida, Enterococcus* sp. and *Enterococcus faecalis*. An overview of the isolated species of aerobic bacteria before and after gingival flushing with 1% povidone-iodine is shown in Table 1.

In the CH group, the bacterial species isolated after rinsing were: *Enterococcus* sp., β-hemolytic *Streptococcus* sp., *Klebsiella* sp. and *Leuconostoc* sp. An overview of isolated species of aerobic bacteria before and after antiseptic gingival flushing with 0.4% chlorhexidine gluconate is presented in Table 2.

In the saline control group, the most commonly isolated bacterial species after rinsing were: *P. multocida, S.aureus, Micrococcus* sp., *E. coli*, β-hemolytic *Streptococcus* sp., *Enterococcus* sp. and *Proteus* sp. An overview of isolated species of aerobic bacteria before and after gingival flushing with saline is given in Table 3.

For the swabs taken before rinsing the mandibular gingiva, 95.6% were positive for cultures, while only two smears were negative (4.4%). All negative pre-flush samples remained negative post-flushing and no sam-

TABLE 3: *Overview of aerobic bacteria identified from the mandibular gingiva prior to and following flushing with saline (SL). Data are presented as number of positive swabs of total 15 (percent).*

Bacteria	Pre-flush SL	Post-flush SL
Pasteurella multocida	8/15 (53.3 %)	4/15 (26.6 %)
Staphylococcus aureus	7/15 (46.6 %)	3/15 (20 %)
Micrococcus sp.	4/15 (26.6 %)	3/15 (20 %)
Escherichia coli	2/15 (13.3 %)	2/15 (13.3 %)
β-hemolytic *Streptococcus* sp.	2/15 (13.3 %)	2/15 (13.3 %)
Enterococcus sp.	2/15 (13.3 %)	2/15 (13.3 %)
Proteus sp.	2/15 (13.3 %)	2/15 (13.3 %)
Bergeyella zoohelcum	2/15 (13.3 %)	1/15 (6.6 %)
Neisseria sp.	2/15 (13.3 %)	0
Staphylococcus pseudintermedius	2/15 (13.3 %)	1/15 (6.6 %)
Acinetobacter sp.	1/15 (6.6 %)	1/15 (6.6 %)
Proteus mirabilis	1/15 (6.6 %)	1/15 (6.6 %)
Stomatococcus mucilaginosus	1/15 (6.6 %)	1/15 (6.6 %)
Pasteurella canis	1/15 (6.6 %)	1/15 (6.6 %)
Streptococcus faecalis	1/15 (6.6 %)	0
Bacillus cereus	1/15 (6.6 %)	0
Leuconostoc sp.	1/15 (6.6 %)	0
Enterococcus faecium	1/15 (6.6 %)	0
Moraxela sp.	1/15 (6.6 %)	0
Klebsiella sp.	1/15 (6,6 %)	0

TABLE 4: *Overview of results of flushing gingiva with PI, CH and SL. In groups PI and CH, negative pre-flush cultures with no growth (BGS=0) were excluded (n=14). Data are presented as number of positive swabs (percent).*

	PI	CH	SL
Positive growth pre-flush	14/15 (93.3%)	14/15 (93.3%)	15/15 (100%)
Positive growth post-flush	3/14 (21.4%)[a]	3/14 (21.4%)[a]	12/15 (80%)[b]
Reduction of ≥1 BGS	13/14 (92.9%)[a]	12/14 (85.7%)[a]	7/15 (46.7%)[b]
Post-flush BGS >1	1/14 (7.1%)[a]	2/14 (14.3%)[a]	8/15 (53.3%)[b]

[a,b] different letters show statistically significant difference between groups (p<0.05)
BGS – bacterial growth score; PI – 1% povidone-iodine; CH – 0.4% chlorhexidine gluconate; SL – 0.9% saline control

ples increased in bacterial growth score post-flushing. Number of animals with positive growth after flushing, number of animals with reduction of bacterial growth score ≥1 and number of animals with bacterial growth score >1 were not different between PI and CH groups. However, number of animals with positive growth after flushing and number of animals with bacterial growth score >1 were lower in PI and CH groups compared to SL group (p<0,05) and number of animals with reduction of bacterial growth score ≥l was higher in PI and CH groups compared to group SL (p<0,05) (Tab. 4).

A summary of the intensity of bacterial growth on the mandibular gingiva prior to flushing is given in Table 5. The differences in the distribution of the level of scoring of the total bacterial growth score prior to flushing was not significant among groups (p=0.7). Table 6 shows the intensity of bacterial growth following gingival flushing and its correlation with the use of a certain antiseptic or saline. Asymptotic Generalized Pearson Chi-Squared Test for ordinal variables showed a significant association between the groups and bacterial growth scores (p<0.001). Post-hoc pairwise ordinal independence test showed a significant difference between PI and SL

TABLE 5: *Intensity of bacterial growth on the mandibular gingiva in all three groups of dogs, prior to flushing. Data present the number of dogs from which swabs were taken from mandibular gingiva prior to flushing.*

Intensity of bacterial growth	GROUPS			total
	PI	CH	SL	
0	1	1	0	2
1	0	1	4	5
2	1	0	0	1
3	2	1	1	4
4	11	12	10	33

Statistical tests showed no significant association between the treatment groups and BGS scores (p=0,7)

TABLE 6: *The intensity of the bacterial growth on the mandibular gingiva post-flushing with povidone-iodine, chlorhexidine gluconate, and with saline solution. Data indicate the number of dogs from which the swabs were taken from the mucosa of the gingival post-flushing.*

Intensity of bacterial growth	GROUPS			total
	PI[a]	CH[a]	SL[b]	
0	12	12	3	27
1	2	1	4	7
2	0	1	0	1
3	1	1	2	4
4	0	0	6	6

[a,b] different letters show statistically significant difference between groups (p<0.05)

groups (p=0,02), as well as CH and SL groups (p=0,02), but not between PI and CH groups (p=0,8).

Discussion

Chlorhexidine gluconate and povidone-iodine are the most commonly used antiseptics in human and veterinary surgery (Lambrechts et al. 2004, Osuna et al. 1990a, b, Swaim et al. 1991). Chlorhexidine is an antiseptic with a broad spectrum of activity, low toxicity and is minimally affected by the presence of organic debris. Its antimicrobial activity is primarily directed at vegetative forms of gram-positive and gram-negative bacteria, though it also has virucidal and fungicidal activity. In relatively low concentrations, chlorhexidine acts as a bacteriostatic, while in higher concentrations it becomes bactericidal and has highly residual activity (Boothe 2001, Lemarie and Hosgood 1995).

Povidone-iodine is also an antiseptic with a wide antimicrobial range and is efficient in contact with a variety of bacteria, viruses, fungi and protozoa. Its extended, non-selective and antimicrobial activity makes it one of the most efficient antiseptics in treating mixed infections (Durani and Leaper 2008). The concentration of chlorhexidine gluconate of 0.4% (200 µg/ml), used in this study, was selected based on the manufacturer's recommendations (Pliva, Zagreb, Croatia). Regarding the concentration of chlorhexidine gluconate and its efficiency on oral bacterial microflora, Tomas et al. (2008) recommend using concentrations of this antiseptic greater than 100 µg/ml (0.2%).

The concentration of povidone-iodine of 1% used in this study was selected based on the manufacturer's recommendations (Alkaloid, Skopje, F.Y.R. Macedonia), based on the bactericidal activity of the 1% povidone-iodine against *S. aureus*. This concentration has shown efficacy against *S. aureus* in vitro in as little as 15 seconds, and against *Mycobacterium chelonei* in as little as 2 minutes (Berkelman et al. 1982). Concentrations lower than 1% have resulted in significantly greater survival of *S. aureus* (Sanchez et al. 1988).

Of the 14 positive swabs before the antiseptic flushing in the PI group, after antisepsis, only three swabs were positive. Potential pathogens identified in the PI post-flush included β-hemolytic *Streptococcus* sp., *P. multocida, Enterococcus* sp. and *Enterococcus faecium*.

Of the 14 positive swabs before antiseptic flushing in the CH group, after antisepsis, only three swabs were positive. Potential pathogens identified in the CH post-flush included β-hemolytic *Streptococcus* sp., *Enterococcus* sp., *Klebsiella* sp. and *Leuconostoc* sp.

As opposed to these two groups, in the control group treated only with saline solution, prior to flushing of the gingiva of the lower jaw, 15 swabs were positive, and after the flushing 12 swabs were positive. The three negative swabs were a result of the mechanical flushing of the gingiva with saline. Potentially pathogenic bacteria isolated and identified from the gingiva of the lower jaw in the SL group were: *Bergeyella zoohelcum, P. multocida, Proteus* sp., *Staphylococcus pseudintermedius, Stomatococcus mucilaginosus,* β-hemolytic *Streptococcus* sp., *Acinetobacter* sp., *Micrococcus* sp., *E. coli, S. aureus, Enterococcus* sp., *Proteus mirabilis*.

The bacterial species identified following flushing of the gingiva: β-hemolytic *Streptococcus* sp., *P. multocida, Enterococcus* sp., *Enterococcus faecalis, Klebsiella* sp., and *Leuconostoc* sp., are normal bacterial microflora in the mouths of healthy dogs. These bacteria are part of the normal bacterial microflora of the canine skin (Scott et al. 2001), and part of the normal bacterial microflora in the canine preputial cavity (Neihaus et al. 2011). Potential pathogenic bacteria may be causes of various infections, including SSI (Giacometti et al. 2000). When identified intraoperatively, certain bacteria have been associated with a higher chance of subsequent wound infection (Twum-Danso et al. 1992).

Preoperative antisepsis cannot be expected to result in complete sterility of the surgical site, and therefore, it is recommended that an antiseptic be selected which will result in the greatest bacterial reduction with the lowest risk of adverse reactions. The results of this study confirmed the hypothesis that the antiseptics povidone-iodine and chlorhexidine gluconate are effective at the tested concentrations and halted bacterial growth on the mucosa of the mandibular gingiva in dogs. No significant differences were found between these two antiseptics in their ability to reduce bacterial growth upon application.

Study Limitations

Quantitative plate counting is considered the gold standard for bacterial quantification; however, semi-quantitative bacterial culture techniques have been validated in studies on IV catheter contamination and surgical site infections (Bouza et al. 2004, 2005, Brodersen et al. 2007, Slobbe et al. 2009). The simplicity of the semiquantitative technique makes it the procedure of choice for routine work in the microbiology laboratory (Bouza et al. 2005).

No antiseptic inactivator was used in this study. The effect of inactivators may be negated during periods of storage or transport, making their use of questionable value when samples are not immediately inoculated (Reichel et al. 2008). In the current study, swabs were plated between 18 and 20 hours after collection, and the authors elected to flush mandibular gingiva with saline to dilute any residual antiseptic to levels that were not bactericidal. Saline rinsing following antiseptic flushing is not routinely performed in the clinical setting, although bacterial growth would no longer be affected beyond the inherent residual effect of the antiseptic.

Only the immediate effect of both antiseptics was studied, while the duration of residual activity is unknown. In this study, the authors chose to only evaluate aerobic bacterial growth due to difficulties in culturing anaerobic organisms and their unknown clinical significance.

Conclusions

Antisepsis of the gingiva with 1% povidone-iodine or with 0.4% chlorhexidine gluconate is significantly effective in reducing bacterial growth. The obtained results suggest that the antiseptics povidone-iodine and chlorhexidine gluconate are a good choice in the concentrations applied, and they are safe for use in preparation of the surgical site. No significant differences were found between 1% povidone-iodine and 0.4% chlorhexidine gluconate in the reduction of bacterial growth on the mandibular gingiva in dogs. Based on the results of this study, a 2-minute flush with 1% povidone-iodine or 0.4% chlorhexidine gluconate is recommended for presurgical preparation of the mandibular gingiva in dogs.

Conflict of interest

We have no conflict of interest to declare.

Ethical approval

The project underwent ethical review and received approval by the institutional (FVMUZ) ethical committee (03-54/1). The owners approved procedure.

Funding

The article was privately funded (PD-PhD dissertation).

Authors contribution

Study conception and design: PD, DV, AM. Acquisition of data: BH, GK, MV. Analysis and interpretation of data: MV, DM, PD, GK. Drafting of manuscript: AM, PD. Critical revision: DV, BH.

References

Beal MW, Brown DC, Shofer FS (2000): The effects of perioperative hypothermia and the duration of anesthesia on postopera-

tive wound infection rate in clean wounds: a retrospective study. Vet Surg 29: 123–127.

Berkelman RL, Holland BW, Anderson RL (1982): Increased bactericidal activity of dilute preparations of povidone-iodine solutions. J Clin Microbiol 15: 635–639.

Bjurstrom L, Linde-Forsberg C (1992): Long-term study of aerobic bacteria of the genital tract in stud dogs. Am J Vet Res 53: 670–673.

Boothe HW (2001): Disinfectants, antiseptics and related germicides. In: Boothe DM (ed.), Small Animal Clinical Pharmacology and Therapeutics. WB Saunders, Philadelphia, USA; 250–257.

Bouza E, Burrilo A, Munoz P, Cercenado E, Rodriquez-Creixems M (2004): Semiquantitative culture of open surgical wounds for diagnosis of surgical site infection. Eur J Clin Microbiol Infect Dis 23: 119–122.

Bouza E, Alvardo N, Alcala L, Sanchez-Conde M, Perez MJ, Munoz P, Martin-Rabadan P, Rodriquez-Creixems M (2005): A prospective, randomized and comparative study of 3 different methods for the diagnosis of intravascular catheter colonization. Clin Infect Dis 40: 1096–1100.

Brodersen HP, Beckers B, Clauss M, Vom Dahl J, Floege J, Janssen U (2007): Identification of central venous haemodialysis catheter-related infection by a semiquantitative culture method. J Nephrol 20: 462–467.

Durani P, Leaper D (2008): Povidone-iodine: use in hand disinfection, skin preparation and antiseptic irrigation. Int Wound J 5: 376–387.

Eugster S, Schawalder P, Gaschen F, Boerlin P (2004): A prospective study of postoperative surgical site infections in dogs and cats. Vet Surg 33: 542–550.

Giacometti A, Cirioni O, Schimizzi AM, Del Prete MS, Barchiesi F, D'Errico MM, Petrelli E, Scalise G (2000): Epidemiology and Microbiology of Surgical Wound Infections. J Clin Microbiol 38: 918–922.

Lambrechts NE, Hurter K, Picard JA, Goldin JP, Thompson PN (2004): A prospective comparison between stabilized glutaraldehyde and chlorhexidine gluconate for preoperative skin antisepsis in dogs. Vet Surg 33: 636–643.

Lemarie RJ, Hosgood G (1995): Antiseptics and disinfectants in small animal practice. Comp Cont Educ Pract Vet 17: 1339–1351.

Neihaus SA, Hathcock TL, Boothe DM, Goring RL (2011): Presurgical antiseptic efficacy Chlorhexidine diacetate and Povidone-iodine in the canine preputial cavity. J Am Anim Hosp Assoc 47: 406–412.

Osuna DJ, DeYoung DJ, Walker RL (1990a): Comparison of three skin preparation techniques in the dog. Part I: Experimental Trial. Vet Surg 19: 14–19.

Osuna DJ, DeYoung DJ, Walker RL (1990b): Comparison of three skin preparation techniques. Part 2: Clinical trial in 100 dogs. Vet Surg 19: 20–23.

Ramos-Vara JA, Beissenher ME, Miller MA, Johnson GC, Pace LW, Fard A, Kottler SJ (2000): Retrospective study of 338 canine oral melanomas with clinical, histologic, and immunohistochemical review of 129 cases. Vet Pathol 37: 597–608.

Reichel M, Heisig P, Kampf G (2008): Pitfalls in efficacy testing – how important is the validation of neutralization of chlorhexidine digluconate? Ann Clin Microbiol Antimicrob 7: 20–26.

Sanchez IR, Nusbaum KE, Swaim SF, Hale AS, Henderson RA, McGuire JA (1988): Chlorhexidine diacetate and povidone-iodine cytotoxicity to canine embryonic fibroblasts and *Staphylococcus aureus*. Vet Surg 17: 182–185.

Scott DW, Miller WH, Griffin CE (2001): Bacterial skin diseases. In: Scott DW, Miller WH, Griffin CE (eds.), Muller and Kirk's Small Animal Dermatology. 6th ed. Elsevier Saunders, Philadelphia; 274–335.

Slobbe L, El Barzouhi A, Boersma E, Rijnders BJA (2009): Comparison of the roll plate method to the sonication method to diagnose catheter colonization and bacteremia in patients with long-term tunneled catheters: a randomized prospective study. J Clin Microbiol 47: 885–888.

Swaim SF, Riddell KP, Geiger DL, Hathcock TL, McGuire JA (1991): Evaluation of surgical scrub and antiseptic solutions for surgical preparation of canine paws. J Am Vet Med Assoc 198: 1941–1945.

Tomas I, Cousido MC, Tomas M, Limeres J, Garcia-Caballero L, Diz P (2008): In vivo bactericidal effect of 0,2% chlorhexidine but not 0,12% on salivary obligate anaerobes. Arch Oral Biol 53: 1186–1191.

Twum-Danso K, Grant C, Al-Suleiman SA, Abdel-Khader S, Al-Awami MS, Al-Breiki H, Taha S, Ashoor AA, Wosornu L (1992): Microbiology of postoperative wound infection: a prospective study of 1770 wounds. J Hosp Infect 21: 29–37.

Vasseur PB, Levy J, Dowd E (1988): Surgical wound infection rates in dogs and cats. Data from a teaching hospital. Vet Surg 17: 60–64.

Weese JS (2008): A review of post-operative infections in veterinary orthopaedic surgery. Vet Comp Orthop Traumatol 21: 99–105.

Weese JS, Halling KB (2006): Perioperative administration of antimicrobials associated with elective surgery for cranial cruciate ligament rupture in dogs: 83 cases (2003–2005). J Am Vet Med Assoc 229: 92–95.

Whittem TL, Johnson AL, Smith CW (1999): Effect of perioperative prophylactic antimicrobial treatment in dogs undergoing elective orthopedic surgery. J Am Vet Med Assoc 215: 212–216.

Address for correspondence
Dražen Vnuk, MS, PhD, prof.
Clinic of Surgery, Orthopedics and Ophthalmology
Faculty of Veterinary Medicine
University of Zagreb
Heinzelova 55
10000 Zagreb
Croatia
dvnuk@vef.hr