PAPER

Comparison of polyhexanide, cold atmospheric plasma and saline in the treatment of canine bite wounds

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Objectives: To compare the efficacy of polyhexanide, cold argon plasma and saline in reducing bacterial bio-burden in dog bite wounds.

Materials and Methods: Prospective blinded randomised clinical trial. Dogs were randomly assigned to one of the treatment groups by lottery and bacterial cultures obtained before and after treatment were compared. Bite wounds were surgically debrided and treated with polyhexanide, cold argon plasma or saline lavage. All wounds were cultured three times: directly after debridement, directly after prelavage with 2 mL/cm² (saline in the saline and cold argon plasma group, or polyhexanide) and following the definitive lavage. Data were analysed using a generalised linear model for ordinal data.

Results: A total of 85 dogs were enrolled in this study (polyhexanide n=29, cold argon plasma n=28, saline n=28). Positive bacterial culture results after debridement were obtained in 53/85 (62.3%) wounds. Polyhexanide and saline lavage significantly reduced the bio-burden, while cold argon plasma treatment did not. This effect was evident after prelavage when polyhexanide performed significantly better than saline and cold argon plasma as well as after final treatment. No significant differences were detected after prelavage or main treatment between saline and cold argon plasma.

Clinical Significance: Polyhexanide lavage achieved the best immediate and ultimate decontamination of bite wounds.

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INTRODUCTION

Bacterial contamination can occur in approximately 48.4% of bite wounds, with 6% of the bacteria classified to be multidrug resistant (MDR) (Nolff *et al.* 2016). More recent prospective investigations have even detected contamination rates as high as 87.5%, with 19.8% of all patients being affected by MDR bacteria (Winter *et al.* 2018). While rising MDR rates have led to investigation of wound antiseptics in human medicine (Kramer *et al.* 2004, Assadian 2007, Daeschlein 2013), research regarding wound antisepsis in veterinary patients is sparse. The use of chlorhexidine in small animal surgery dates back to the works of Lozier *et al.* (1992) and Sanchez *et al.* (1988). This recommendation contrasts with the current recommendations in human medicine (Kramer *et al.* 2004, 2018, Assadian 2007, Daeschlein 2013). Most importantly, resistance against chlorhexidine has been documented and may even induce cross-resistance against different antibiotic classes, including macrolides and vancomycin (Willy 2013, Beier *et al.* 2015, Bhardwaj *et al.* 2016). Understandably, newer antiseptic alternatives such as polyhexanide-biguanide and physical treatments have gained increasing interest in human medicine (Kramer *et al.* 2004, 2018, Assadian 2007, Daeschlein 2013).

Recently, the combination of polyhexanide-biguanide was approved by the food and drug administration and licensed as a wound antiseptic in the USA (Eberlein & Assadian 2010). The

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substance belongs to the biguanide group (as does chlorhexidine) and the proposed mechanism of action is binding and disruption of negatively charged membrane acid phospholipids (gram negative bacteria), teichoid acids (gram positive bacteria) and peptidoglycans (Kaehn 2010). Recent work by Chindera *et al.* (2016) found energy-dependent cell uptake of the chemical, causing cell elongation and chromosome condensation – a mechanism that has not been described so far for any other drug. In mammalian cells the substance is trapped in the endosomes and thus causes no harm (Ikeda *et al.* 1983, 1984, Kaehn 2010, Wessels & Ingmer 2013, Chindera *et al.* 2016). Due to this selective effect, polyhexanide is bactericidal with a high therapeutic index, displays a sustained postantiseptic effect, and is neither affected by blood nor proteins within the wound (Kramer *et al.* 2004, Müller & Kramer 2008, Eberlein & Assadian 2010, Kaehn 2010, Müller *et al.* 2013). While polyhexanide has been used in canine dermatologic indications (Mills *et al.* 2005), to our knowledge there are currently only very few veterinary reports available that investigated the effect of this modern wound antiseptic for wound decontamination (Nolff *et al.* 2015, Winter *et al.* 2018).

Among the physical antibacterial treatment options, cold plasma is one of the most highly investigated alternatives (Von Woedtke *et al.* 2013). Plasma is the fourth state of matter (solid, liquid, gas, plasma), and different effluents can be created depending on gas species, gas pressure and the amount of added energy. A mixture of electromagnetic radiation including infrared, visible and ultraviolet light, reactive oxygen and nitrogen species such as OH radicals, ozone and electric currents are created in the effluent at a tolerable temperature of between 38 and 63°C (Von Woedtke *et al.* 2013, Uchiyama *et al.* 2015). Although the exact mechanism of action is not fully understood, numerous *in vitro* studies have proven the high antibacterial efficiency of these systems (Daeschlein *et al.* 2010, 2012c, 2012d, 2015, Bender *et al.* 2011, Matthes *et al.* 2016). In addition to the *in vitro* studies, initial *in vivo* trials also showed a profound wound decontamination effect (Hammann *et al.* 2010, Daeschlein *et al.* 2012a, 2012b, Heinlein *et al.* 2013). So far, veterinary descriptions of plasma for wound decontamination are limited to the pilot study of this project, published in 2018 by Winter and colleagues. The pilot project was performed in order to allow a sample size calculation for this current follow-up study and was underpowered to detect statistical significance of treatment. Furthermore, it was an intention-to-treat design, so duration of cold argon plasma treatment as well as of polyhexanide application was not controlled.

The aim of this current study was to compare the decontaminating performance of polyhexanide, cold argon plasma and saline lavage in dog bite wounds. The study design (including treatment duration and volume) and sample size calculations were based on the results of a pilot study (Winter *et al.* 2018) that was completed before the start of the current study. No patients of the pilot study were included in the current study. Our hypothesis was that both polyhexanide and cold argon plasma treatment would result in greater bacterial bio-burden reduction than saline lavage.

MATERIALS AND METHODS

The study was performed after gaining approval of the ethics commission of our facility (38-20-12-2014) after completion of the pilot study between January and June 2015 (Winter *et al.* 2018). The results of this pilot project were used for initial evaluation of practicability of the treatments, as well as for sample size calculation. Both studies are independent from each other and were undertaken in sequence. Dogs were included if they had been presented to the clinic due to witnessed acute bite wound injuries without any prior surgical treatment between June 2015 and July 2017, and if a complete follow-up was available until suture removal. All patients were prospectively evaluated regarding signalment, injury location and type, bacterial bio-burden and effect of antiseptic treatment after debridement and randomly assigned to one of three treatment groups by lottery (polyhexanide-biguanide (ProntoVet®, B.Braun) cold argon plasma (kinPEN®VET, NeoPlas) or 0.9% saline (NaCl, B.Braun). The plasma device used in this study (kinPEN®VET) was an atmospheric plasma jet with a handheld unit and consisting of a 1-mm pin tip electrode mounted in the centre of a quartz capillary (1.6 mm inner diameter, Fig. 1). Argon was selected as a working gas at a flow of 4.5 standard L/minute. The gas was ignited at the tip of the electrode and created a jet-like effluent covering approximately 1 cm^2. At these settings the effluent had a visible length of 14 mm and a constant temperature of 48°C at the tip. The treatment surface was scanned at a distance of approximately 1 cm in a circular pattern.

Wound treatment

Patients were anaesthetised after stabilisation as needed (IV crystalloid fluids, electrolyte correction if necessary). The skin surrounding the wounds was aseptically prepared and washed using

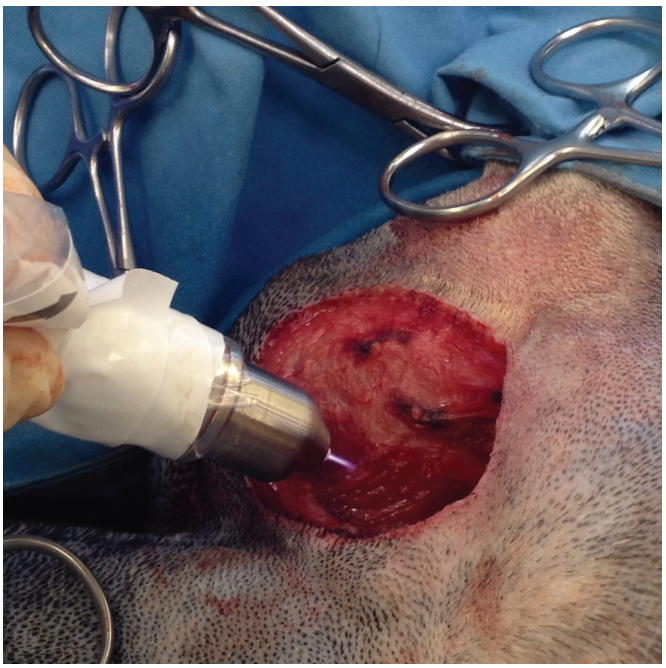

FIG 1. Showing the CAP device during treatment. Note the ignited gas at the tip of the pen

iodine solution (Jodosept® PVP, Vetoquinol GmbH) and disinfected using alcohol (Softasept® N, B.Braun) while the wounds were covered with dry gauze. After preparation, all patients were transferred into the same operating room. Bacterial bioburden in the wounds was measured at three different time-points (baseline, after prelavage with a fixed volume of lavage solution of 2 mL/cm^2 wound area and no contact time, and finally after main lavage and a controlled soak time of 15 minutes after the lavage). The intention was to allow individual differentiation between the effect of time and lavage volume on total decontamination.

The wounds were surgically debrided, and the first culture swab was obtained by evenly rolling the swab over the entire wound surface, avoiding contact with the surrounding skin directly after debridement (sterile transport swab, Sarstedt AG & Co or Transystem®, Hain Lifescience GmbH). In cases with abdominal or thoracic perforation, the body cavity was closed after debridement, before the first swab was taken and lavage was performed. The size of the wounds was determined after debridement and before taking the first swab by measuring the wound length and width with a sterile ruler.

Prelavage with a defined volume of 2 mL/cm^2 was then performed as follows: in the saline and polyhexanide group the wound was rinsed with the assigned substance using low pressure (application using a syringe without a needle); in the cold argon plasma group the wound was prelavaged with saline. A second swab was taken directly after the used substance was fully drained from the wound surface after prelavage. Finally, the main lavage was performed using the substances of interest. Saline and polyhexanide were applied onto the wounds out of the container, with sterile gauze placed in the wound at the end of the lavage to enable a soak time of 15 minutes (Eberlein & Assadian 2010). The gauze was then removed after the 15 minutes were completed and the swab was taken from the moist wound surface as described before. For cold argon plasma treatment the maximum treatment time was set at 15 minutes, wounds that were smaller than 7.5cm^2 were treated in overlapping circles for 2 minutes/cm^2. After this, a third swab was obtained from the moist wound surface as described above. The volume used for the final lavage was at the discretion of the attending surgeon, and was recorded. The treatment volume was determined by subtracting the non-used volume from the total volume. The wounds were then closed using monofilament resorbable suture (polydioxanone, MonoPlus® B.Braun) with a Penrose drain inserted which exited at the ventral-most point adjacent to all wounds before closure. The wound and drain were covered by medical adhesive drape (Cutiplast, Smith&Nephew). The wound location, wound size (cm^2) as well as surgery time (initial incision until removal of surgery drapes), anaesthesia time (induction – extubation) and main lavage volume were documented.

Microbiological assessment

The culture swabs were routinely investigated by an accredited institute and bacterial species (maldi-TOF) as well as number of cultured bacteria (semi-quantitative score; (+)=growth after enrichment=category 1, +=1 to 10 CFU=category 2, ++ 11 to 100 CFU = category 3, +++ >100 CFU=category 4) were determined. Phenotypic antibiotic resistance was assessed according to Clinical and Laboratory Standards Institute (CLSI VET01 document) guidelines. Based on these results a summed contamination score was calculated for each time point by adding the semi-quantitative score of all detected isolates per swab (*e.g.* a wound with a ++ *Escherichia coli* (Bellingieri *et al.* 2016) plus (+) *Staphylococcus* (Assadian 2007) would result in a contamination score of 4 (Table S1, Supporting Information displays the distribution of summed scores at each sampling point).

All isolates were tested for susceptibility to the following: doxycycline, sulfonamide-trimethoprime, amoxicillin-clavulanic acid, cefalothin, cefovecin, nitrofurantoin, enrofloxacin, marbofloxacin, gentamicin, imipenem, ampicillin and amikacin. MDR was defined as described by Gandolfi-De Christophoris *et al.* (2013); detection of resistance (including intermediate effect; ECDC guidelines; European Centre for Disease Prevention and Control 2014) to three or more major antibiotic classes.

Follow-up

Body temperature and wound healing parameters (swelling, reddening, heat and discharge from suture line or drainage exit) of all patients were evaluated the day after surgery. All animals were hospitalised as long as the drains were in place. Drains were removed on the third day after surgery or, if there was no discharge in the drape covering the drainage site in the 12 hours after drape exchange. The wound was checked finally at scheduled suture removal (at 10 days – either in our clinic or by the referring veterinarian). All complications detected during hospitalisation were recorded and graded into minor [no intervention (medical or surgical needed) and major (surgical intervention needed, death of patient)]. Complications recorded at suture removal included need for surgical revision or death. If revision was required because of infection the bacteria identified at the time of complication were compared to the results of the initial surgery.

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

Treatment Modality	Polyhexanid	Saline	CAP
Number of dogs included (n)	29	28	28
Mean wound area cm^2 (sd)	63.5 (±107.2)	34.2 (±48.9)	26.8 (± 36.1)
Mean surgery time in minutes (sd)	53.6 (±37.9)	61.43 (±55.4)	40.13 (±19.3)
Mean anaesthesia time in minutes (sd)	98.3 (±45.8)	61.3 (±55.4)	95.8 (±28.4)
Mean volume main lavage in ml (sd)	311.6 (±176.9)	1090 (±816.1)	–
Mean duration of argon treatment in minutes (sd)	–	–	13.8 (±2.7) (30.9 seconds/cm^2)

Statistics

A sample size analysis was performed using G*Power 3.1 software. The parameters were based on the results of the pilot study (Effect size of $d = 0.8$, alpha error 0.05, power 0.8) resulting in a minimum required sample size of 28 *per* group. Statistical analysis was performed using SPSS® Statistics. Descriptive statistics were obtained, and all data were tested for normality using the Kolmogorov-Smirnov test. Intra- and intergroup comparisons of decontamination for each treatment were compared using a generalised linear model for ordinal data, with the significance level set at $P<0.05$.

RESULTS

Patient data

A total of 90 patients were initially included in this study: five were lost to follow-up resulting in a final group distribution of polyhexanide treatment, n=29; cold argon plasma treatment, n=28; and, saline, n=28. Altogether, 28 different breeds were included (Table S1). The mean age of patients was 5.7 months [(polyhexanide 5.7 (±4.1) months, saline 6.1 (±3.4) months, cold argon plasma 5.4 (±2.6)] months, the mean weight was 17.3 kg [(polyhexanide 16.2 (±8.9) kg, saline 16.5 (±3.4), cold argon plasma 19.1 (±10.6)]. The majority of included patients (57%) were male (72% of these intact).

Wound data

The most frequently affected location was the extremities (23/85), followed by the thoracic wall (16/85, two perforating), neck (15/85), lateral and ventral abdomen (13/85, three perforating), back (10/85) and the perineum (1/85). The majority of patients were presented within 8 hours after injury (9/85 within the first hour, 43/85 between 1 and 8 hours after injury, 19 within 8-24 hours after injury and 14/85 more than 24 hours after injury). One patient had received antibiotics from the referring veterinarian. All patients underwent surgery within 6 hours after initial presentation to our clinic. The mean overall wound size after debridement was 41.7 cm^2 (±72.6 cm^2), on average, the size of the underlying wounds was 2.8 times bigger compared to the outer perforation of the skin. Mean anaesthesia time was 99.3 minutes (±46.3 min), mean surgery time was 51.7 minutes (± 40.7 min). Further details are given in Table 1. Mean duration of hospitalisation was 2.8 days (±2.3).

Culture results

A total of 53 of 85 (62.3%) patients had positive bacterial culture results after debridement. MDR were isolated in 21 of 53 patients with positive initial culture results (39.6%). Table 2 shows the susceptibilities of individual bacteria classed as being MDR.

A total of 45 different bacterial subspecies were detected. The following bacterial groups were detected most frequently (more than two isolates detected in first swab): *Pasteurella* species (n=42), *Streptococcus* species (n=27), *Staphylococcus* species (n=17), *Neisseria* species (n=17), *Pseudomonas* species (n=5) and

Table 2. Bacteria isolates being classed as MDR and susceptibility to the tested antibiotics

Isolate	Total	DOX	TSO	AMX	PEN/AMP	CEF 1	CEF 3	NIT	STR	GEN	PRA	MAR	ENR	CLIN/LINC	ERY	CHL	COL	Isolates with detected MDR
*Escherichia coli**	2			1	2	2	2		1					2	2			2/2
Enterococcus faecalis	2					1	1	1	1	1	1	1	1	1	1	1	1	1/2
Pasteurella like bacteria	6						1		2					2				1/6
Pasteurella canis	21			1		1			7	2				20	11		1	4/21
Pasteurella dagmatis	11		2						4	1		1	1	9	2			2/11
Pasteurella multocida	2								1					2	1			1/2
Pasteurella stomatis	2		1						1	1		1	1	1				1/2
Neisseria species	5												1	3		1		1/5
Pseudomonas aeruginosa	5	5	5	5	5	5	5	5	3		4		4	5	5	5		5/5
Staphylococcus pseudintermedius	11	3	7	2	9			1	4					5	4	3	9	5/11
Proteus mirabilis	1	1						1						1	1		1	1/1
Streptococcus canis	5	5	2						5	1	1	4	5	1	2	1	5	5/5

Antibiotics that were identified to be non-effective (resistant) in bacteria isolated in the first wound swab; total total number of isolates of this bacteria-subgroup in swab 1, MDR multidrug-resistance, DOX doxycycline, TSO sulfonamide trimethoprim, AMX potentiated amoxicillin/clavulanic acid, PEN/AMP penicillin/ampicillin, CEF1 cefalothin/cephalexin (first generation), CEF3 cefovecin (third generation), NIT nitrofurantoin, STR streptomycin, GEN gentamycin, PRA pradofloxacine, MAR marbofloxacine, ENR enrofloxacine, CLIN/LINC clindamycin/lincomycin, ERY erythromycin, CHL chloramphenicol, COL colistin

Table 3. The distribution, median and range of contamination scores (CS) achieved in the included wounds at all time points. The score was calculated by adding the severity factor of each individual bacterium encountered in the wound

	Polyhexanide				Saline				Cold Argon Plasma		
Study ID	S1	S2	S3	Study ID	S1	S2	S3	Study ID	S1	S2	S3
1	**3**	**0**	**0**	6	**5**	**2**	**0**	4	**1**	**0**	**0**
2	0	2	0	8	**6**	**4**	**0**	20	2	1	2
3	**3**	**0**	**0**	10	9	11	7	21	**2**	**0**	**0**
5	0	0	1	11	**3**	**0**	**0**	26	7	1	1
7	0	0	0	14	**3**	**0**	**0**	27	**2**	**2**	**0**
9	**6**	**0**	**0**	15	**1**	**0**	**0**	28	8	8	7
12	**27**	**0**	**0**	16	0	0	0	29	9	1	2
13	**12**	**0**	**0**	18	**3**	**0**	**0**	35	0	0	0
17	0	0	0	22	0	0	0	43	**2**	**0**	**0**
19	**3**	**0**	**0**	23	0	0	0	46	0	0	0
25	0	0	0	24	0	0	0	49	0	1	0
37	0	0	0	30	1	1	1	50	20	10	13
39	0	0	0	31	0	0	0	51	4	6	5
40	0	0	0	34	9	9	9	52	0	0	0
42	**3**	**0**	**0**	36	0	0	0	55	0	1	0
48	0	0	0	38	0	0	0	56	0	0	2
53	**2**	**0**	**0**	45	2	2	1	57	4	3	3
54	0	0	0	47	**2**	**1**	**0**	59	3	2	2
58	0	0	0	65	6	6	1	61	0	0	2
63	0	0	0	66	0	0	0	62	0	0	0
64	8	6	2	68	4	5	11	75	6	9	9
67	**2**	**0**	**0**	69	0	0	0	83	3	3	2
70	**1**	**0**	**0**	72	1	1	1	84	10	4	7
71	0	0	0	74	6	5	5	85	**2**	**0**	**0**
73	**2**	**0**	**0**	76	12	7	3	86	4	4	4
78	7	6	3	77	8	7	1	88	6	3	1
79	0	0	0	80	0	0	0	89	2	2	3
81	**15**	**7**	**0**	87	1	1	1	90	**1**	**0**	**0**
82	**4**	**0**	**0**								
Median	1	0	0		1.5	1	0		2	1	2
range	0 to 27	0 to 7	0 to 3		0 to 12	0 to 11	0 to 11		0 to 20	0 to 10	0 to 13
Full Decont.	13/15 (86.6%)				7/18 (38.8%)				6/20 (30%)		

Full. Decomp wounds initially tested positive that became negative under therapy, these are marked bold

Corynebacterium (n=3) (further information on the individual subspecies is given in Supplementary Table S2).

There were no statistical differences between groups regarding the contamination score after initial debridement (saline *versus* polyhexanide P=0.548; polyhexanide *versus* cold argon plasma P=0.634), saline *versus* cold argon plasma P=0.287) (Table 3). Polyhexanide treatment resulted in a highly significant decrease of the summed wound contamination score over time (P=0.001), as did saline lavage (P=0.037). CAP did not achieve a significant bioburden reduction over time (P=0.199). Intergroup comparisons after prelavage showed that polyhexanide performed significantly better than saline prelavage (P=0.006). After the main lavage (mean volume of 4.9 mL/cm^2 wound area for polyhexanide and mean volume of 31.9 mL/cm^2 wound area for saline) followed by a soak time of 15 minutes polyhexanide still performed significantly better than saline (P=0.018). Additional treatment using cold argon plasma (mean 30 seconds/cm^2 wound area) after saline prelavage did not result in a further decrease in wound bioburden (Table 3). Direct comparison between cold argon plasma and the other treatment groups after final treatment showed that there was no significant difference detected between cold argon plasma and saline treatment (P=0.109) while polyhexanide performed significantly better than cold argon plasma (P<0.001).

Table 4. The percentage of patients *per* group affected by at least 1 MDR isolate and the complication rate per group

Substance	Polyhexanide	CAP	Saline
Overall complication rate	8/29	6/28	8/28
MDR rate	4/18	8/20	9/15

Complications

During surgery, the treatment of wounds using cold argon plasma frequently led to disturbance of electromagnetic signals, with electrocardiogram monitoring being particularly affected. These interferences were only detected during treatment and did not cause any further complications. Minor complications (incisional discharge, swelling) were detected in nine of 85 cases (10.6%). Major complications occurred in 13 of 85 (15.3%) patients – of these, nine patients required additional surgery: seroma formation (n=1), wound infection (n=2), skin necrosis (n=3), dehiscence (n=1), retained Penrose drain (n=1) and development of an indolent pocket wound (n=1). Three patients had positive cultures at the time of revision: *Staphylococcus aureus* (n=1), *Staphylococcus pseudintermedius* (n=3). All of these had cultured negative from the last swab during initial surgery. Four patients died (mortality rate

4.7%); three due to sepsis and one dog was euthanased because of ongoing infection). Three of the dogs that died had injuries to the thoracic wall (one penetrating) and one had a (non-penetrating) abdominal wall injury. All of these patients were less than 15 kg bodyweight. Neither the type of bacterium nor the presence of MDR could be correlated with the occurrence of major complications (Table 4).

DISCUSSION

We chose canine bite wounds to test for bacterial decontamination effect because they represent a frequent source of contaminated traumatic wounds in veterinary patients (Shamir *et al.* 2002). The documented contamination rate of bite wounds lies between 48% and 95.8%, with a wide variety of different bacteria encountered (Kelly *et al.* 1992, Griffin & Holt 2001, Meyers *et al.* 2008, Mouro *et al.* 2010, Nolff *et al.* 2016). In the current study 62.3% of the patients were presented with contaminated wounds and the bacterial species identified generally resembled previously published data (Kelly *et al.* 1992, Griffin & Holt 2001, Meyers *et al.* 2008, Mouro *et al.* 2010, Nolff *et al.* 2016). A total of 39.6% of patients were affected with at least one isolate classified to be MDR. This MDR rate was substantially higher than the MDR rate of 6% published by Nolff *et al.* 2016, which had been recorded between 2010 and 2014, as well as the rate reported by Winter *et al.* 2018 (19.8%). This is a concerning finding, which highlights the need for improved antibiotic stewardship programmes and the need for alternative methods for bacterial decontamination besides standard antibiotic therapy.

The data support our hypothesis that polyhexanide treatment results in more effective wound decontamination than saline lavage, with a significant increase of the effect over time, underlining the beneficial effect of an appropriate contact time. A retrospective open-label controlled multicentre randomised cohort study involving 7862 human patients with severely contaminated soft tissue injuries previously documented that the infection rate was lowest in patients treated with polyhexanide compared to PVP-iodine, ringer or hydrogen peroxide (Roth *et al.* 2007). We now show that polyhexanide also exerts a superior decontamination effect when compared with saline and cold argon plasma in dogs. Although saline also achieved a significant decrease of contamination score over time, polyhexanide significantly outperformed saline at both time-points (after prelavage as well as after definite treatment). One limitation is the potential residual activity and the consequence of this on potential results of microbiological culture (Payne *et al.* 2018). While residual antiseptic activity is a phenomenon that is desirable under clinical conditions (Kramer *et al.* 2004, Müller & Kramer 2008, Eberlein & Assadian 2010, Kaehn 2010, Müller *et al.* 2013), it might have impacted our results because of ongoing bacterial destruction after the swab was taken. Due to this effect, other authors have inactivated the polyhexanide after samples were taken in clinical trials in humans (Payne *et al.* 2018). This procedure was not followed in the current study and we can therefore not ultimately prove whether the wounds or just the swabs were decontaminated. However, since Payne *et al.* (2018) also found that polyhexanide significantly outperformed saline, we consider it unlikely that inactivation would have influenced our final results to a great degree. In addition, compared to our pilot study, where contact time and structured lavage were not controlled for, we were now able to double the number of patients in which complete decontamination was achieved from 41% in the pilot study to 86.6% in the current study by implementing a structured treatment protocol. This indicates that the residual effect is most likely not the only explanation for the superior performance of polyhexanide in the current study, as it did not improve the outcome in the pilot project. Instead this strongly suggests the importance of a structured and controlled treatment protocol for application of wound antiseptics. However, further studies investigating the effect of antagonisation of culture swabs in clinical cases after treatment with wound antiseptics are needed to solve this potential bias. This might also change the way we handle microbiological cultures under clinical circumstances in general.

In contrast to the performance of polyhexanide, we had to reject our hypothesis that additional cold argon plasma treatment after prelavage with saline would improve wound decontamination. Indeed, additional cold argon plasma treatment did not seem to exert any decontamination effect at all. This is astonishing, because several studies have investigated cold argon plasma in comparison with other antiseptics in humans and rodents (chlorhexidine, polyhexanide), and reported that it was superior (Hammann *et al.* 2010, Koban *et al.* 2011, Matthes *et al.* 2014, Bellingieri *et al.* 2016). One possible explanation might be the setup of the plasma source. As the KinPen®Vet only allows treatment of approximately 1 cm^2 at a time, there is no possibility to treat the whole wound at once. Plasma treatment is time-dependent, with the greatest effects described for treatment times between 10 seconds *per* cm^2 and 5 minutes in total (Daeschlein *et al.* 2010, 2012b, 2012c, 2012d, 2015, Bender *et al.* 2011, Heinlein *et al.* 2013, Matthes *et al.* 2016). Due to the wound size, we only reached a mean treatment time of 30 seconds *per* cm^2 if we limited total treatment to a maximum of 15 minutes, which might not have been enough. Furthermore, while moving over the wound area, cross-contamination of treated areas by bordering non-treated areas might be of concern. We are not able to fully describe the reason for the great discrepancy between previous experimental and clinical studies and our study, but most of them used bigger plasma sources, with the capacity to treat the whole wound area at once. The drawback of large plasma sources is the cost and immobility, which was the reason why the portable plasma pen used in this study has been developed. However, based on the results of this study, we cannot recommend its usage for decontamination of bite wounds in dogs.

A major limitation of the study is the mode of evaluation of bacterial bio-burden. Despite taking great care to include the entire wound surface, our evaluation is only semi-quantitative. No tissue biopsies were taken, and no attempt was made to accurately quantify the bacterial load. Ideally, the whole treated surface should be probed (excisional biopsy) and evaluated using quantitative methods such as qPCR. However, since this was a

clinical study, taking additional biopsies was not considered to be ethically justifiable.

We were not able to detect any impact of residual contamination score or presence of MDR bacteria on complications, but our general complication rate was low and therefore correlations between residual wound bio-burden and occurrence of complications should be evaluated with caution. The clinical impact of the residual bacteria within the wound at the time of closure remains unclear.

In conclusion, polyhexanide lavage achieved the best immediate and ultimate decontamination of bite wounds followed by saline and is thus recommended as the lavage solution of choice in clinical cases of bite wounds. Cold argon plasma treatment using a portable plasma pen did not add any decontamination effect after saline prelavage. The bacteria identified resemble those found in previous studies on bite wound contamination. Worryingly, we detected a higher proportion of MDR isolates than previously reported. This further underlines the fact that alternative antibacterial strategies need to be investigated.

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Conflict of interest

The authors state that there is no conflict of interest.

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Supporting Information

The following supporting information is available for this article:

Table S1. Distribution of individual breeds within the study population

Table S2. Distribution of the different bacteria-subspecies after debridement in the study population