Bacterial isolation and evaluation of antisepsis protocols of the operative field of bitches submitted to ovariohysterectomy

Isolamento bacteriano e avaliação deprotocolos de antissepsia do campo operatório de cadelas submetidas àovariohisterectomia

Sabrina Cândido Trajano[1]* iD, Breno Bezerra Aragão[1] iD, Mauro de Araujo Penaforte Junior[1] iD, Kleyton Domingos de Melo[1] iD, Maria Sheila da Silva Ferreira[1] iD, Júnior Mário Baltazar de Oliveira[1] iD, Rinaldo Aparecido Mota[2] iD & Grazielle Anahy de Sousa Aleixo[2] iD

[1]Veterinarians, MSc. Programa de Pós-graduação em Medicina Veterinária – PGVET, Universidade Federal Rural de Pernambuco – UFRPE, Recife, PE, Brasil
[2]Veterinarians, DSc. Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco – UFRPE, Recife, PE, Brasil

Abstract

This study evaluated the efficiency of five antisepsis protocols performed in the operative field of bitches due to the importance of preventing surgical infections in veterinary medicine. Thirty female bitches submitted to elective ovariohysterectomy (OVH) were used and were separated into groups of eight animals. In group I, 70% alcohol and 2% chlorhexidine gluconate (CHG) were administered; in group II, alcoholic CHG 0.5%; in group III, polyvinylpyrrolidone (PVPI) 10% alcohol; in group IV, alcohol 70% and alcoholic PVPI 10%; and in group V, alcohol, PVPI 10%, and CHG degermante 2%. Samples were collected with sterile swabs before antisepsis, and after 3 min, each product was used and seeded in a specific medium for colony forming unit (CFU) counts. The identification of the isolates was performed according to the morphological, dyeing, and biochemical characteristics, namely, *Bacillus* spp., *Staphylococcus* spp., *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Shigella sonnei*, *Shigella* spp., *Enterobacter aerogenes*, *Enterobacter* spp., *Salmonella* spp., and *Escherichia coli*. The results obtained allow us to conclude that the protocols with 0.5% CHG and 2% CHG degermante were the most efficient in antisepsis that reduced 100% of the CFU of the skin and presented better residual power until the end of the surgical procedure.

Keywords: antisepsis protocol, *Cannis familiaris*, elective surgery, surgical site.

Resumo

Este estudo avaliou a eficácia de cinco protocolos de antissepsia realizados no campo operatório de cadelas, devido à importância da prevenção de infecções cirúrgicas em medicina veterinária. Foram utilizadas 30 cadelas submetidas à ovariohisterectomia eletiva (OVH), divididas em grupos de oito animais. No grupo I, foram administrados álcool 70% e gluconato de clorexidina 2% (CHG); no grupo II, CHG alcoólico 0,5%; no grupo III, polivinilpirrolidona (PVPI) álcool a 10%; no grupo IV, álcool 70% e PVPI alcoólico 10%; e no grupo V, álcool, PVPI 10% e CHG degermante 2%. As amostras foram coletadas com "swabs" estéreis antes da antissepsia e, após 3 minutos, cada produto foi utilizado e semeado em meio específico para contagem de unidades formadoras de colônias (UFC). A identificação dos isolados foi realizada de acordo com as características morfológicas, de tingimento e bioquímicas, a saber, *Bacillus* spp., *Staphylococcus* spp., *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Shigella sonnei*, *Shigella* spp., *Enterobacter aerogenes*, *Enterobacter* spp., *Salmonella* spp. e *Escherichia coli*. Os resultados obtidos permitem concluir que os protocolos com 0,5% CHG e 2% CHG degermante foram os mais eficientes na antissepsia que reduziram 100% das UFC da pele e apresentaram melhor poder residual até o final do procedimento cirúrgico.

Palavras-chave: protocolo de antissepsia, *Cannis familiaris*, cirurgia eletiva, sítio cirúrgico.

Introduction

Surgical interventions include the occurrence of contamination and/or alterations causing disorders in the patient, such as pain, discomfort, and stress, in addition to increasing returns to the veterinary hospital for new treatments (Stehling et al., 2001).

Infection of the surgical site (SSI) can affect organs or structures approached during the surgical procedure and can manifest itself between a period of 30 days to one year after surgery in cases of using implants (Poveda et al., 2003). Despite advances in hospital infection control practices in recent years, SSI is still among the leading causes of morbidity and mortality in patients in the postoperative period (Sparling et al., 2007; Singh & Weese, 2017).

Until the middle of the 19th century, surgical infections were a major obstacle to the evolution and development of surgery (Dellinger, 2003). Before the discovery of microorganisms, antisepsis, and their importance in infection, surgical procedures were performed in a bloody manner and with no prospect of patient survival being the same, as the processes were performed by so-called barber surgeons (Thorwald, 2005; Amato, 2006). Since discovery of antisepsis methods in the middle of the 19th century, surgery went through a great evolution (Dellinger, 2003).

Antisepsis of the patient's skin is one of the main measures to prevent SSI. For this, several antiseptic products have been developed over the years to prevent and reduce the risk of infection (Phillips et al., 1991). The ideal antiseptic should have a fast action, broad antimicrobial spectrum, and prolonged residual action, act in the presence of blood and secretions, does not interfere with tissue healing, in addition to being hypoallergenic, non-teratogenic, and not mutagenic (Cunha et al., 2008; Moriya & Módena, 2008; Agência Nacional de Vigilância Sanitária, 2010). The products most used in skin antisepsis are 70% alcohol, 10% polyvinylpyrrolidone (PVPI), and chlorhexidine gluconate (CHG), which can be found in various concentrations and in aqueous, alcoholic, and degerming formulations (Moriya & Módena, 2008).

It is known that the microbiota of the skin of dogs and cats is formed by two types of microorganisms, namely, the transient and resident bacteria. The transient are recent environmental microorganisms that are non-colonizing and survive for a short period on the skin, such as Gram-negative bacteria, including *Escherichia coli*. Resident bacteria, on the other hand, have a greater resistance and have a longer life span on the skin. They are, therefore, considered as colonizers, such as *Staphylococcus* spp. and *Corynebacterium* spp. (Murray, 1995).

To evaluate the importance of preventing surgical infections in veterinary medicine, this study aimed to highlight the efficiency of five antisepsis protocols performed on bitches undergoing ovariohysterectomy (OVH) by using 70% alcohol solution, 2% chlorhexidine, 0.5% alcoholic chlorhexidine, and 10% alcoholic povidone iodine alone or in combination, so that the bacteria present on the skin of patients could be isolated and identified.

Materials and methods

This study was approved by the Ethics Committee on the Use of Animals of the referred institution, under protocol number 107/2017.

Forty healthy bitches, admitted for the elective OVH surgical procedure, were used, with no predilection for race. The animals were aged between one and six years, with body weight between seven and 13 kg. The patients underwent a clinical and surgical risk assessment, where the following preoperative examinations were performed: blood count; biochemical with serum dosage of the enzymes alanine aminotransferase; aspartate transaminase; alkaline phosphatase; urea and creatinine; and electrocardiogram and abdominal ultrasound. The dogs — healthy and not pregnant — were subject to OVH surgery.

The bitches were separated into five groups, each comprising eight animals to perform five different antisepsis protocols. In group I, 70% alcohol was used with 2% CHG degermante; in group II, the alcoholic solution of CHG 0.5% was used; in group III, 10% alcoholic PVPI; in group IV, alcohol 70% and alcoholic PVPI 10%; and in group V, alcohol, followed by alcoholic PVPI 10%, and finally CHG degermante 2% was used.

After shaving and removing the free hairs with a sterile gauze pad, a sterile swab was rubbed in a Müeller-Hinton broth solution (a moment that was considered T_0). Thereafter, according to the protocol adopted in each group, antiseptics were applied (one at a time) to the entire area prepared with the aid of sterile gauze pads handled with sterile Colin antisepsis forceps, and another swab was rubbed 3 min after the passage of each antiseptic solution (moments T_1, T_2, T_3, and T_4 depending on the protocol), thus evaluating the isolated action of each product. At the end of the surgery, another Swabian was passed to assess the reduction of the microbiota by

the synergistic action of the solutions. All Swabians were soaked in Müeller-Hinton broth before being passed over the animal's skin.

The application of antiseptic products followed a pattern where each solution was applied once in sufficient quantity to moisten the entire operative field and, it is, then, left to act for 3 min.

The operating room was kept with the doors closed to prevent the entry of people who were not part of the team, thus minimizing the contamination of the environment.

After collection, the tubes with Swabians were identified and sent immediately to the Laboratory of Infectious Contagious Diseases at Universidade Federal Rural de Pernambuco (UFRPE) to perform the processing.

The tubes containing the swab samples were incubated at 35-37 °C for 24 h to reduce the damage caused by antiseptics to microorganisms. After incubation, the samples were diluted in 0.9% saline until a 10^6 dilution was achieved, and then the CFU quantification technique was performed by surface plating on soy tray agar (TSA), enriched with 0.02% lecithin, 5% Tween 80, and sodium thiosulfate. For plating, 1 mL of each dilution was aliquoted and sown in TSA with the aid of Drigalski loops, and the same being performed in duplicate for each dilution obtained. After the inoculation of the samples, the plates were incubated at 35-37 °C for 24 h, and after bacterial growth, the CFU/mL count was performed (Silva et al., 2000).

The percentages of the CFU count reduction were calculated for each sample of the five protocols, using the formula given below (Osuna et al., 1990; Silva et al., 2000):

$$\text{Immediate bacterial reduction } (\%) = \frac{\text{CFU post} - \text{trichotomy } - \text{ CFU post} - \text{antisepsis } \times 100 \ (\text{T1})}{\text{CFU post} - \text{trichotomy}} \tag{1}$$

$$\text{Reduction of bacteria at the end of surgery } (\%) = \frac{\text{CFU post} - \text{trichotomy } - \text{ CFU end of surgery } \times 100 \ (\text{T2})}{\text{UFC post} - \text{trichotomy}} \tag{2}$$

The Swabians were also seeded on Base Agar plus 5% sheep blood and Methylene Blue Eosin Agar (EMB LEVINE), using the streak depletion technique and subsequently incubated at 35-37 °C for 24-48 h. The isolation of bacteria was obtained at different collection times. After this period, the colonies were read and Gram stained to determine the morphology of the bacterial agents (Stinghen et al., 2002).

After the morphotintorial evaluation of the Gram test, the bacterial isolates were inoculated in tubes containing broth *Brain Heart Infusion* at 35-37 °C for 24 h. Subsequently, biochemical tests for the identification of bacterial isolates began, where for isolates with characteristics of the genus *Staphylococcus*, coagulase and catalase tests were performed (Silva et al., 2017), and for the other agents, the Adolfo Lutz Institute (IAL) medium was used (Pessoa & Silva, 1972).

After the stages of microbiological analysis of quantification, isolation, and identification of microorganisms, an antimicrobial resistance test was carried out.

The data obtained were subjected to analysis of the distribution of values (normality) using the Shapiro-Wilk test. Afterwards, the values of the UFC reduction rates obtained in the groups were compared using the Mann-Whitney nonparametric test (Sampaio, 1998). The IBM SPSS Statistics 23.0 program was used to perform statistical calculations with a significance level of 5.0%.

Results

Bacterial count

The total number of samples collected from each animal varied according to the protocol adopted in each group. In groups I and IV, four samples were collected from each dog, in groups II and III, three samples, and in group V, five samples. The transoperative period varied between 16 and 21 min, but the time required to perform antisepsis varied according to the protocol used, varying from 3-9 min.

Bacterial growth was observed in all Swabian samples collected before antisepsis was performed (T_0); counting was possible only at -5 and -6 dilutions due to the high level of contamination.

Group I (70% alcohol + 2% CHG) showed intense bacterial growth in all TSA plaques before and after alcohol use, i.e., 100% of the bitches (T_1); however, after using CHG degerming 2% (T_2), the bacterial count decreased to zero and remained until the end of the surgical procedure (T_3). In group II, the use of 0.5% alcoholic CHG (T_1) resulted in a 100% bacterial reduction after antisepsis, and there was no bacterial growth until the end of the procedure (T_2).

In group III (alcoholic PVPI at 10%), bacterial growth was observed in 25% of bitches (2/8) at T_1 and 62.5% (5/8) at the end of the surgical procedure (T_2). In group IV (alcohol 70% + PVPI, 10%), bacterial growth was observed in 100% (8/8) of the animals after the use of 70% alcohol (T_1), 25% (2/8) after using alcoholic PVPI 10% (T_2) and 50% (4/8) at the end of the surgery (T_3). Group V (alcohol 70% + alcoholic PVPI 10% + CHG degermante 2%) showed bacterial growth - 100% (8/8) at T_1 and 50% (4/8) at T_2 (after using PVPI); however, there was no bacterial growth after the use of CHG degermante 2% (T_3) or at the end of the surgery (T_4) (Table 1).

Table 1. Rate of bacterial growth of samples collected at different times.

Time of collection	Group I n (%)	Group II n (%)	Group III n (%)	Group IV n (%)	Group V n (%)
T0	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)
T1	8 (100%)	0 (0%)	2 (25%)	8 (100%)	8 (100%)
T2	0 (0%)	0 (0%)	5 (62.5%)	2 (25%)	4 (50%)
T3	0 (0%)	-	-	4 (50%)	0 (0%)
T4	-	-	-	-	0 (0%)

n= number of animals; T0= berfore the antiseptic solution; Group I, T1= alcohol 70%, T2= CHG 2%; T3= after surgery; Group II, T1= CHG 0,5%, T2= after surgery; Group III, T1= PVPI 10%, T2= after surgery; Group IV, T1= alcohol 70%, T2= PVPI 10%, T3= after surgery; Group V, T1= alcohol 70%, T2= PVPI 10%, T3=CHG 2%, T4= after surgery.

Regarding the efficiency of the antiseptic protocol, the protocols used in groups I, II, and V showed 100% bacterial reduction 3 min after using the last solution (chlorhexidine degermante 2%) and without bacterial growth until the end of surgery. The least efficient protocols were used in groups III and IV, respectively (Table 2).

Table 2. Comparison between the reduction rates of UFC accessories in the groups.

Groups	Average rate of reduction of CFU[1]
1	100.0 ± 0.0a
2	100.0%a
3	75.5 ± 36.3b
4	68.9 ± 52.0b
5	100.0 ± 0.0a*

*Mean and standard deviation; [1]Mann-Whitney test; Different letters in the same column indicate statistical difference (p <0.05) between groups.

During the surgical procedure, an acute allergic reaction was observed in 5% of the dogs (2/40) after the application of 10% alcoholic PVPI. Within 30 days after surgery, no patient had clinical signs suggestive of SSI, such as redness, increased local temperature, pain, and secretion at the incision site.

Identification of isolates

Most of the isolates were obtained from skin swabs passed at T_0, i.e., before performing antisepsis, corresponding to 63.8% (76/119). These isolates were: *K. pneumoniae* (9/119), *K. oxytoca* (5/119), *S. sonnei* (4/119), *Shiguella* spp. (1/119), *E. aerogenes* (16/119), *Enterobacter* spp. (1/119), *Salmonella* spp. (1/119), *E. coli* (2/119), coagulase-positive *Staphylococcus* (10/119), *Bacillus* spp. β-hemolytic (26/119), and *Bacillus* spp. non-hemolytic (4/119) (Table 3).

Table 3. Identification and percentage of bacteria isolated in samples of skin swabs from bitches submitted to elective ovariohysterectomy (OVH).

Bacteria Gram negativas	n (%)
Klebsiella pnemoniae	9 (7.5%)
Klebsiella oxytoca	5 (4.2%)
Shigella sonnei	4 (3.3%)
Shigella spp.	1 (0.8%)
Enterobacter aerogenes	17 (14.3%)
Enterobacter spp.	1 (0.8%)
Salmonela spp.	1 (0.8%)
Escherichia coli	2 (1.6%)
Bacteria Gram positivas	**n (%)**
Staphylococcus coagulase +	16 (13.5%)
Bacillus spp. β hemolítico	55 (46.2%)
Bacillus spp. não hemolítico	8 (6.7%)

Twenty strains (16.8%) were isolated after the use of 70% alcohol in groups I, IV, and V: *E. aerogenes* (1/119), *K. oxytoca* (1/119), coagulase-positive *Staphylococcus* (2/119), *Bacillus* spp. β-hemolytic (13/119), and *Bacillus* spp. non-hemolytic (3/119). After PVPI, were identified coagulase-positive *Staphylococcus* (3/119), *Bacillus* spp. hemolytic β (8/119), and *Bacillus* spp. non-hemolytic (1/119), corresponding to 10% (12/163). The remaining isolates were obtained from swabs passed at the end of the surgical procedure, corresponding to 8.4% (10/163), namely, *Bacillus* spp. β-hemolytic (9/119) and coagulase-positive *Staphylococcus* (1/119). Even with the use of PVPI, it was possible to isolate bacteria of the genus *Bacillus* spp. (β-hemolytic) and *Staphylococcus* spp. in groups III and IV and *Bacillus* spp. β-hemolytic in group V (Table 4).

Table 4. Bacteria isolated from cutaneous swabs in the surgical field of bitches submitted to elective ovariohysterectomy (OVH) at different times called T_0, T_1, T_2, T_3, and T_4.

Bacteria	Group I				Group II			Group III			Group IV				Group V					Total
	T0	T1	T2	T3	T0	T1	T2	T0	T1	T2	T0	T1	T2	T3	T0	T1	T2	T3	T4	
Klebsiella pnemoniae	2*	-	-	-	2	-	-	2	-	-	-	-	-	-	3	-	-	-	-	9/119
Klebsiella oxytoca	2	-	-	-	2	-	-	-	-	-	-	1	-	-	-	-	-	-	-	5/119
Shigella sonnei	-	-	-	-	-	-	-	1	-	-	2	-	-	-	1	-	-	-	-	4/119
Shigella spp.	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1/119
Enterobacter aerogenes	2	1	-	-	2	-	-	4	-	-	5	-	-	-	3	-	-	-	-	17/119
Enterobacter spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1/119
Salmonella spp.	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1/119
Staphylococcus coagulase +	2	2	-	-	3	-	-	2	1	1	1	-	2	-	2	-	-	-	-	16/119
Bacillus spp. β-hemolítico	3	2	-	-	5	-	-	6	2	3	6	5	3	6	6	6	2	-	-	55/119
Bacillus não hemolítico	2	2	-	-	-	-	-	-	-	-	-	1	1	-	1	1	-	-	-	8/119
Escherichia coli	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2/119

Discussion

Bacterial count

All antisepsis methods showed some degree of efficiency in reducing the CFU count of the samples of skin swabs; however, the protocols in which the solutions of 0.5% alcoholic CHG (group II) and 2% alcoholic CHG (groups I and V) were the most efficient, with no CFU formation 3 min after application and at the end of the surgical procedure. This demonstrates the efficiency of this solution in surgical antisepsis, which prevented the formation of CFU and showed residual action until the end of the procedure, maintaining its antibacterial activity even in the presence of blood, as has already been reported in other studies (Moriya & Módena, 2008; Agência Nacional de Vigilância Sanitária, 2010). In addition, the use of CHG alone for 3 min does not require the use of 70% alcohol and/or 10% alcoholic PVPI, reducing the time and cost of antisepsis solutions.

Although alcohol is considered the safest antiseptic due to its low toxicity and bactericidal effect (Agência Nacional de Vigilância Sanitária, 2010), its use in groups I, IV, and V was not efficient, allowing bacterial growth in all animals for 3 min after its use. The efficiency of alcohol may have been reduced due to the presence of bacterial spores that germinated when inoculated in TSA plates, since most of the isolates that grew after their use were *Bacillus* spp., a large spore producer. In addition, alcohol may have exerted action only on transitory bacteria, as resident bacteria are present in the deeper layers of the skin, presenting greater resistance (Bond & Loeffler, 2012).

According to Boyce & Prittet (2002) and Burg et al. (2007), when an emollient product is added to alcohol, its bactericidal action is improved, as its volatile capacity decreases, allowing the product to remain in contact with the skin for a longer time, despite continuing to offer no residual effect and not to destroy spores. For this, it must be used as an alcoholic preparation (PA) in association with CHG or PVPI (Moriya & Módena, 2008; Agência Nacional de Vigilância Sanitária, 2010; Gonçalves et al., 2012). Despite the recommendations, the results obtained did not show 100% efficiency in reducing CFU when alcoholic PVPI was used.

It is known that the action of iodine is effective for 1-2 min and has a residual effect of 2-4 h; however, this was not observed in this study, even though the time required for the action of the product was respected as recommended (Agência Nacional de Vigilância Sanitária, 2010). The low efficiency of iodine can be explained by its inactivation in the presence of blood. PVPI can also be inactivated in the presence of inorganic compounds such as alcohol; however, in this study, alcohol did not interfere with the action of PVPI because in group III alcohol was not used before PVPI and even then, it showed low efficiency (Cunha et al., 2008; Moriya & Módena, 2008; Agência Nacional de Vigilância Sanitária, 2010).

An immediate allergic reaction to PVPI was observed in two dogs in this study. The same occurred in a study carried out by Osuna et al. (1990), where they mentioned that 50% of the areas prepared with this solution had an acute allergy. PVPI causes less skin irritation than iodine; however, it causes more contact dermatitis than other antiseptic solutions such as CHG. A major negative factor of iodine present in PVPI is its affinity for organic matter, and it also reduces its efficiency Due to this, new alternatives should be sought (Agência Nacional de Vigilância Sanitária, 2010).

Rodrigues (1997) suggested the use of CHG as an alternative to replacing PVPI in patients allergic to iodine, which occurred in two dogs in the current study. CHG is poorly absorbed by intact skin, and if percutaneous absorption occurs, it becomes insignificant. However, CHG has no indication for use in antisepsis in some areas such as the ear and eye region due to its ototoxic activity, which may cause eye damage (Larson, 1988). In addition, it can also slow down the healing process and cause flaking in the tissues (Agência Nacional de Vigilância Sanitária, 2010).

The results showed that there was no significant difference between the use of 0.5% alcoholic CHG and 2% dehydrating CHG, as both reduced the UFC count by 100% 3 min after use and maintained residual action until the end of the surgical procedure. This result was similar to other studies where CHG was used in the antisepsis of dogs and cats (Osuna et al., 1990; Silva et al., 2000; Marchi et al., 2018). Alcoholic CHG 0.5% is easier and more practical to apply to the skin, with no need to remove the excess product, as it evaporates, leaving the skin dry, unlike what happens with CHG degermante, where it is necessary to remove the excess product.

As in this study, CHG has also been shown to have superior antiseptic activity when compared to PVPI. In a study by Darouiche et al. (2010), the group that received antisepsis with CHG 0.5%

had less infection than the group where PVPI was used. Other authors have reported the use of chlorhexidine to combat outbreaks of *S. aureus*, including those that have methicillin resistance in intensive care units in human hospitals through daily baths in adults and newborn children (Christensen et al., 2001; El Helali et al., 2005; Sandri et al., 2006; Cunha et al., 2008). While Nogueira et al. (1999) reported similar antimicrobial activity between CHG and PVPI suggesting that both are ideal for surgical antisepsis of the abdominal operative field of cats with an action time of five min; however, the authors do not mention product concentrations.

Some criteria must be followed for the choice of antiseptics, such as the spectrum of antimicrobial activity, speed of action, residual power, low toxicity, good performance in the presence of organic matter, and not interfering in the wound healing and repair process (Osuna et al., 1990; Phillips et al., 1991; Schulz, 2014). Stubbs et al. (1996) indicated that CHG is the antiseptic of choice, as it fulfills the desired qualifications for an antiseptic solution. In this study, dogs that used CHG degermante 2% and alcoholic CHG 0.5% in the antiseptic protocol did not present an acute allergic reaction, had good healing of the surgical wound and, in addition, had excellent bactericidal action and residual effect, being these antiseptic solutions that best fit the characteristics recommended for an ideal antiseptic.

Identification of isolates

Regarding the isolates, Gram-positive bacteria were the most frequent (66.4%); however, the majority were of the genus *Bacillus* spp. (52.9%), which are environmental bacteria that have no clinical importance. The other 13.5% were isolated from the *Staphylococcus* genus, all of which showed a positive reaction in the coagulase and catalase tests. Similar studies identified a greater number and also identified a greater number of Gram-positive bacteria on the skin of dogs and cats (Ishii et al., 2011; Murta et al., 2015).

Coagulase-positive *Staphylococcus* species can behave opportunistically (Frank et al., 2003; Morris et al., 2006), which can cause serious infections in the postoperative period of these animals (Oliveira et al., 2006; DeLeo & Chambers, 2009). Studies show the transmission of opportunistic pathogens such as coagulase-positive *Staphylococcus* (SCP) between humans and dogs, including species that have multidrug resistance, such as *S. aureus* and *S. pseudointermedius*, the first being considered an anthropozoonosis, where transmission probably occurs in humans (Tomlin et al., 1999). This makes the animals in this research in which *Staphylococcus* isolates become a risk group for SSI.

Regarding the action of antiseptic solutions on bacterial isolates, alcoholic CHG 0.5% and CHG degermanent 2% eliminated 100% of the isolates, which is different from what was described in a study carried out by Monteiro et al. (2001), in which they reported bacterial growth in 20% of the dogs after antisepsis with CHG degermante 2%. Alcohol 70% before the use of CHG degermante 2% in group I, and alcoholic PVPI 10% in group IV did not interfere with their action.

The use of alcohol in groups I, IV and V (at time T1) had a varied action when comparing Gram-positive and Gram-negative bacteria, where it was observed that in relation to Gram-positive bacteria, the germicidal action was not significant because the number of bacterial strains isolated before the use of 70% alcohol had a reduction of only 21% after the use of the product (5/23). Regarding Gram-negatives, there was a significant rate of colony reduction of 91.3% (21/23). Alcohol is characterized by having a fast action against Gram-positive and Gam-negative bacteria; however, in this study, it did not show fast action and did not show efficiency against Gram-positive bacteria, as reported in the literature. To improve its antiseptic capacity, the literature recommends using alcohol in PA with CHG or PVPI, as occurred in group II (Boyce & Prittet, 2002; Moriya & Módena, 2008; Gonçalves et al., 2012).

The 0.5% alcoholic CHG PA used in group II bitches' antisepsis eliminated 100% of Gram-negative and Gram-positive bacteria, preventing bacterial growth until the end of the surgical procedure. Strains of SCP and *Bacillus* spp. β-hemolytics that were resistant to 70% alcohol and 10% alcoholic PVPI were sensitive to 0.5% alcoholic CHG, in agreement with Stubbs et al. (1996), who reported that CHG has an action against *Staphylococcus* genus bacteria and is therefore used to control outbreaks of *S.aureus*, including those that are resistant to methicillin in intensive care units in human hospitals, through daily baths and intranasal use in adults and newborn children (Christensen et al., 2001; El Helali et al., 2005; Sandri et al., 2006; Cunha et al., 2008).

The use of alcohol and PVPI was not sufficient to eliminate bacterial flora in groups III, IV, and V, in which it was possible to isolate Gram-positive bacteria after their use. The 10% alcoholic PVPI solution was also used in group V together with the 2% CHG degerming agent. After its use, the remaining microorganisms from alcohol and PVPI were eliminated, and the 2% CHG degerming solution that prevented bacterial growth until the end of the surgical procedure was more efficient than alcoholic PVPI 10%.

Even with the use of PVPI, it was possible to isolate bacteria such as *Bacillus* spp. β-hemolytic and coagulase-positive *Staphylococcus* in groups III and IV and *Bacillus* spp. β-hemolytic in group V, which is a producer of spores, according to Sagripanti & Bonifacino (1996) and McDonnell & Russell (1999). The spores of bacteria of the genus *Bacillus* are resistant to different types of antiseptics, including PVPI.

Bacterial growth at the end of surgery in the groups where the PVPI solution was used as a part of the antiseptic protocol (groups III, IV and V) demonstrated that this solution did not present a good residual action, since the surgical procedure lasted between 16 and 21 min (short duration) and bacterial growth of *Staphylococcus* spp. and *Bacillus* spp. Considering that there was bacterial growth in a short-term surgical procedure, its use against long-term surgery antisepsis is contraindicated. In a study by Monteiro et al. (2001), the growth of *S. aureus* and *Bacillus* spp. was observed in 40% of the animals after antisepsis with PVPI and growth of *Bacillus* spp. in 20% of dogs using 2% CHG degerming. They associated the growth of Bacillus spp. to the fact that CHG has no action against spores.

In this research, when alcoholic PVPI 10% was associated with CHG degermante 2%, there was no bacterial growth until the end of the surgery; however, we believe that it was the action of CHG degermante 2% that did not allow bacterial proliferation and promoted residual action.

Conclusion

It was concluded that the protocols where 0.5% alcoholic CHG and 2% deodorant were used showed the best antiseptic activity when applied to the skin of bitches that were submitted to elective OVH presented 100% effectiveness in eliminating CFU until the end of the surgical procedure. Due to its practical application on the skin, owing to its bactericidal and residual action, and the fact that it is unnecessary to remove the excess product after use, alcoholic CHG 0.5% is recommended as the antiseptic of choice to perform skin antisepsis in dogs.

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