ORIGINAL ARTICLE

https://doi.org/10.21708/avb.2021.15.2.9801

# Comparative study of the effectiveness and residual effect of preoperative antisepsis with 4% chlorhexidine gluconate associated with 70% ethyl alcohol and 0.5% alcoholic chlorhexidine gluconate in dogs

## *Estudo comparativo da eficácia e do efeito residual da antissepsia pré-operatória com gluconato de clorexidina 4% associado a álcool etílico 70% e gluconato de clorexidina alcoólico 0,5% em cães*

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**ABSTRACT:** The effectiveness of antisepsis of surgical sites in 20 animals (canine species) was compared and subdivided into two groups, using 4% chlorhexidine gluconate associated with alcohol (group 1) and 0.5% chlorhexidine gluconate (group 2). The samples were collected through skin swab after trichotomy (T1), after definitive antisepsis (T2) and one hour after the use of antiseptic (T3), and then submitted to the count of colony forming units (CFU). In both groups, bacterial growth occurred in T1; in T2, the reduction of CFUs was significant for both groups (G1 and G2); however, if we consider absolute values, we can see in T1 a greater amount of CFUs in G2, and when evaluating the results of T2, we can see values which are very similar between G1 and G2, which may suggest greater efficiency of G2 in initial times after antisepsis. In T3, the reduction of CFUs was more effective for G1, suggesting a greater residual effect when compared to G2. Both antiseptic protocols were effective as they significantly reduced the number of skin bacteria, both in T2 and T3.

**KEYWORDS:** antiseptic; surgical field; small animals.

**RESUMO:** A eficácia da antissepsia dos sítios cirúrgicos em 20 animais (espécie canina) foi comparada e subdividida em dois grupos, utilizando gluconato de clorexidina 4% associado ao álcool (grupo 1) e gluconato de clorexidina 0,5% (grupo 2). As amostras foram coletadas por meio de swab cutâneo após tricotomia (T1), após antissepsia definitiva (T2) e uma hora após o uso de antisséptico (T3), sendo então submetidas à contagem das unidades formadoras de colônias (UFC). Em ambos os grupos, o crescimento bacteriano ocorreu em T1; em T2, a redução das UFCs foi significativa para ambos os grupos (G1 e G2); porém, se considerarmos os valores absolutos, podemos observar em T1 uma maior quantidade de UFCs no G2, e ao avaliar os resultados de T2, podemos observar valores que são muito semelhantes entre G1 e G2, o que pode sugerir maior eficiência de G2 em tempos iniciais após a antissepsia. No T3, a redução das UFCs foi mais efetiva para o G1, sugerindo maior efeito residual quando comparado ao G2. Ambos os protocolos antissépticos foram eficazes, pois reduziram significativamente o número de bactérias cutâneas, tanto em T2 quanto em T3.

**PALAVRAS-CHAVE:** antisséptico; campo cirúrgico; animais pequenos.

## INTRODUCTION

Surgical infection is one of the most common and challenging postoperative complications in veterinary medicine; several prophylactic protocols and measures are required to ensure antisepsis and sterility during surgeries (FOSSUM, 2014).

Post-surgical infections should be analyzed according to the potential for contamination of the surgical wound, understood as the probable number of microorganisms found in the tissue to be operated on. Therefore, the surgeries should be classified by the surgeon according to the potential contamination of

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Received: 10/03/2020. Accepted: 12/30/2020

the surgical wound in the following categories: clean, clean-contaminated, contaminated and infected surgeries (ANVISA, 2017; PRATES et al., 2018).

However, all surgical wounds may be deemed contaminated, since microorganisms live naturally on the skin surface of patients, especially in the stratum corneum and inside the sweat glands, sebaceous glands and hair follicles. At the end of the 1990s, the decontamination of living tissues became more relevant, especially for healthcare professionals, when it was determined that the patient is the primary source of infection (RODRIGUES et al., 1997; SILVA et al., 2000). In veterinary medicine, this fact may be even more relevant, because animal patients are not clean and do not receive hygiene care like humans. Thus, it is indispensable that the veterinarian and his/her team carry out prophylactic measures, such as patient antisepsis, to reduce the risk of infections (BELO et al., 2018; FOSSUM, 2014).

For the prophylaxis of surgical infections, it is essential to know risk factors, highlighting the clinical conditions of the patient, preoperative hospital stay, environmental conditions of the surgical room, number of people inside the room, degermation techniques for the surgeon's hands and his/her team, antisepsis of the patient's skin, surgery time, surgical technique and the surgeon's skills (ARIAS et al., 2013; ERCOLE et al., 2011; KRUMMENNAUER; MENEZES; RENNER, 2019).

The discovery of asepsis and antisepsis represents one of the major advances in infection prevention (URQUIZA et al., 2016). Asepsis is a set of practical measures aimed to prevent the contamination in surgery (SILVA et al., 2015) and antisepsis is one of the steps of asepsis, the main goal of which being to reduce or eliminate resident or transient microorganisms from the skin of the area where surgery is performed (LÓPEZ et al., 2017). Thus, it is essential to choose an appropriate antiseptic, which can be classified as bactericidal agents, with the ability to eliminate bacteria in vegetative forms, or as bacteriostatic agents, when they only inhibit the growth of these microorganisms (DIOMEDI et al., 2017; OLIVEIRA; GAMA, 2018).

An ideal antiseptic should offer: a broad spectrum of action; fast action; low toxicity and low inactivation next to organic matter; good residual and cumulative effect; pleasant odor; good acceptance by the user; be stable and non-corrosive, and have affordable cost and great availability in the market (OLIVEIRA; GAMA, 2018).

In Brazil, there are various antiseptics; traditionally, we have alcohol, polyvinylpyrrolidone iodine and chlorhexidine gluconate. However, the most recent studies worldwide have shown greater effectiveness in the use of a chlorhexidine solution associated with 70% ethyl alcohol (AYOUB et al., 2015; EDMISTON et al., 2013).

Ethyl alcohol has concentrations between 60 and 90%, with 70% being more appropriate as it presents better antiseptic effects with lower abrasiveness to the skin, as well as a broad spectrum and fast action, with effectiveness in 15 seconds. However, alcohol does not have a residual effect and becomes inactive in the presence of organic matter, in addition to being volatile and flammable (MASUKAWA et al., 2016).

Ethyl alcohol is a bactericide used against vegetative forms of gram- positive and gram- negative microorganisms, acting in the denaturation of bacterial proteins; it is also a fungicide and virucide used against some viruses; because of this, it is used in the composition of others antiseptics (MORIYA; MÓDENA, 2008; REIS et al., 2011).

Chlorhexidine gluconate is used as an antiseptic for this skin, wounds, mucous membrane and mouth, where it has, at low concentrations, a bacteriostatic effect and a bactericidal effect at high concentrations. The solution acts on the cytoplasmic membrane of the microorganisms promoting its rupture and has a broad spectrum of action, mainly against bacteria (Gram-positive, Gram-negative, aerobic and facultative anaerobic bacteria), viruses and some filamentous and yeast-form fungi (GARCIA, 2017); additionally, it has a good residual effect, with effect action in 15 seconds and its antimicrobial persistence increases when associated with alcohol, therefore becoming a good option for use in patients allergic to iodine. Chlorhexidine is available as a 2 or 4% degerming solution, as a 0.5% alcoholic solution and as a 2.0%, 0.2% and 0.12% aqueous solution (ANVISA, 2016; MASUKAWA et al., 2016).

In the clinical and surgical practice at human hospitals, it is common to use a 0.5% chlorhexidine alcoholic solution as the sole agent for skin antisepsis of patients submitted to clean and clean-contaminated surgeries. However, animals have a large amount of hair and less hygiene than humans, which implies a greater probability of surgical infection in veterinary patients. Thus, in veterinary surgeries the use of a more concentrated solution of chlorhexidine (4%) associated with 70% ethyl alcohol is more common (FOSSUM, 2014). However, there are no data in the literature proving the best effectiveness between one technique and the other. Therefore, we believe that this research aimed to determine, by means of microbiological analyses, which antisepsis protocol of the surgical site, whether 4% chlorhexidine gluconate associated with 70% ethyl alcohol or 0.5% alcoholic chlorhexidine gluconate, is more effective in bacterial control and provides a greater residual effect in clean and clean-contaminated dog surgeries.

## MATERIALS AND METHODS

All the procedures performed were revised and approved by the Animal Use Ethics Committee of the University Center Ingá through authorization PM31/2017.

A preoperative bath was standardized 24 hours in advance for animal surgery, according to surgical and anesthetic recommendations handed over to the owner, ensuring the removal of loose hairs, detritus and external parasites. Hair removal occurred near the time of the surgery and outside the surgical room.

The preparation of the surgical site was performed aseptically by an assistant with a preliminary antisepsis, followed by a final antisepsis performed by the surgeon using sterile tweezers and plugs, making circular movements while rubbing the area, moving them from the center to the peripheral area, discarding the plugs after reaching the peripheral area (FOSSUM, 2014).

The study was carried out at the Veterinary Clinic of the University Center Ingá - UNINGÁ, in which two groups were formed with 10 animals (dogs) each. Hospital routine animals were used, with elective, urgent or emergency surgeries, classified as clean or clean-contaminated. The first collection of samples from the surgical site, using sterile swabs, occurred within the surgical room, after preanesthetic medication and hair removal of the operating field. With the patient in a surgical position, the swab was passed through the skin, called time one (T1). Antisepsis was performed according to one of the two protocols proposed below, one minute later, and the second sample was collected using new swab (time two - T2). The surgery was started immediately, and after one hour a third sample was collected, called time three (T3).

The first group (G1) was submitted to the antisepsis protocol composed of initial degermation with 4% degermante chlorhexidine gluconate, followed by 70% alcohol with three repetitions each, and then, with the surgeon already gowned appropriately, the same protocol was aseptically performed. In the second group (G2), the degermation method followed the same principles, using only 0.5% alcoholic chlorhexidine gluconate. As a standard, sterile gloves were used to collect the material. The animals that received the various products were randomly selected.

After the collections, the swabs were immediately placed into test tubes containing 3 ml of Stuart transport medium and sent to the laboratory of veterinary microbiology.

The samples were then inoculated into Petri dishes with a blood agar culture medium and incubated for 48 hours at 37 °C, counting colony forming units (CFU) 24 and 48 hours after incubation; afterwards, statistical evaluations were performed on these samples using MS Excel.

## RESULTS

After counting the growth bacterial colony forming units in their respective groups and times, the average results are shown in the following table (Table 1).

Thus, growth of a large number of colony forming units (CFU) was seen in all T1 for both groups, while in T2 and T3 there was a decrease of CFU growth for the G1 and G2 groups within 24 hours as well as within 48 hours.

The main bacteria found in the evaluation of the microbiota of the animal's surgical field were: Staphylococcus aureus; Enterococcus sp., Streptococcus sp.

## DISCUSSION

In most species of microorganisms, the infective load or the number of CFUs capable of promoting an infectious process in the individual within a given environment, whether a clinical or hospital environment, is not yet established. Surgical site infections have a complex and multifactorial aspect and may be related to the surgeon, patient and the surgical team (GOULART; ASSIS; SOUZA, 2011). Thus, the study had a comparative focus between antisepsis protocols, determining whether the number of CFUs found was sufficient to cause an infection or not in case of possible contamination during surgery at the surgical site of the animal. However, we can affirm that no animal operated using such methods of antisepsis in the surgical site showed signs of sepsis or contamination of the surgical wound.

When attempting to perform statistical analyses of the data, due to the biological nature of the study, a very significant numerical variation of CFU growth of both protocols was found, resulting in a very high coefficient of variation (CV), the most probable and central factor being the varying degrees of contamination of each animal before each antisepsis. The CV allows for comparisons between distinct variables in terms of nature so as to provide an idea of data accuracy. The smaller the CV, the more homogeneous the data (GARCIA, 1989). The non-use of statistical analysis due to high CV has made the performance of comparisons and results of the data of this study to be carried out by analyzing the CFU averages and their standard deviation, since average comparison tests have been frequently used by researchers (BERTOLDO et al., 2008).

Given the diverse conditions in which pets live, it is clear how complex and uncertain the standardization is in terms of the cleaning these patients received for surgery. In this project, the number of colony forming units (CFU) in the initial samples

**Table 1.** Average value and standard deviation of colony forming units (CFUs) at different times of antisepsis performed in the surgical field of the animal.

| Groups | T1 | | T2 | | T3 | |
|---|---|---|---|---|---|---|
| | 24 hours | 48 hours | 24 hours | 48 hours | 24 hours | 48 hours |
| G1 | 225.9±249.62 | 309.9±405.69 | 0.4±0.96 | 0.7±1.05 | 0.2±0.63 | 0.2±0.63 |
| G2 | 462.3±669.99 | 596.4±868.70 | 0.4±1.26 | 0.7±1.56 | 0.7±1.56 | 0.9±1.59 |

Group G1 = surgical antisepsis of the animal with a protocol composed of initial degermation with 4% degermant chlorhexidine gluconate, followed by 70% alcohol with three repetitions each; Group G2 = antisepsis only using 0.5% alcoholic chlorhexidine gluconate. T1 = Sample analysis before the previous antisepsis of the surgical field; T2 = Sample analysis after the final antisepsis performed by the surgeon; T3 = Sample analysis 1 hour after surgery started.

accounts for these varieties. Of the 20 analyzed samples, the amount of CFU/ml ranged from 0 to 2,151 with an average of 225.9 ± 868.702. In a similar study carried out by Silva et al. (2000), the amount of CFU / ml ranged from 130.00 to 174,000.00 with an average of 26,066.25 ± 49,884.69, corroborating such variation.

Based on this contamination disparity, the protocol suggested here provides for the degermation of the operating field with 0.5% alcoholic chlorhexidine gluconate, performed without surgical gowning, only with procedure gloves aiming at physical cleaning, with the animal duly positioned and properly anesthetized. It is suggested that, under friction with a gauze, the product is spread throughout the operating field, until removal is performed without evidence of soiling. After this step, in a definite way and with the surgeon already gowned appropriately, sterile tweezers and plugs are used and soaked with an 0.5% alcoholic chlorhexidine gluconate antiseptic, making circular movements while rubbing, moving them from the center to the peripheral area, and discarding the plugs after reaching the center. Group 1 (G1), with an initial application of 4% chlorhexidine gluconate as a degerming agent, followed by 70% alcohol with three repetitions each in the preliminary and final antisepsis, was used as a control because its use has already been established (FOSSUM, 2014).

When assessing the T2 period (immediately after antisepsis), the results of both groups (G1 and G2) showed a significant reduction when compared to the initial bacterial load (T1), i.e. proving the efficacy of the two protocols. However, if we take into account the absolute values, in T1 we can see a larger number of CFUs in G2, and when evaluating the results of T2 we can see similar values between G1 and G2, which may suggest greater efficiency of G2 in the initial times after antisepsis. We believe that this occurs due to the aqueous characteristic of the 0.5% chlorhexidine solution, which provides greater "washing" of the surgical site when compared to the 4% chlorhexidine degermant.

When analyzing the number of CFUs after one hour of antisepsis (T3), the 4% chlorhexidine gluconate and 70% alcohol (G1) protocol seems to achieve greater efficacy with a possible residual effect, when compared to the 0.5% chlorhexidine gluconate protocol (G2), since there was greater bacterial growth in G2 when compared to the values of T2 and T3. According to Beraldo; Andrade (2008), chlorhexidine is an antimicrobial agent with a broad spectrum of activity against gram-positive microorganisms, including oxacillin-resistant Staphylococcus aureus, and vancomycin-resistant Enterococcus sp. It is absorbed by the tissues, causing residual effect over time, and may present activity up to five hours after the application. Thus, we believe that the degerming characteristic of 4% chlorhexidine may favor the adhesion of the antiseptic to the skin and provide greater bacterial control in the long term when compared to the aqueous solution of 0.5% chlorhexidine.

In general, the reduced number of CFUs after antisepsis with the use of 0.5% chlorhexidine represents a cheaper protocol alternative, and if percutaneous absorption occurs up to the systemic route, it will be insignificant due to the low concentration of the product (RODRIGUES et al., 1997).

## CONCLUSION

Both antisepsis protocols tested are effective in the immediate elimination of microorganisms from the skin flora. However, after the first hour, the protocol with 4% chlorhexidine gluconate and 70% alcohol seems be more efficient due to its residual effect.

## ACKNOWLEDGEMENTS

We would like to thank the University Center Ingá (Uningá) for the financial and technical collaboration for the development of this work.

## REFERENCES

ANVISA, Agencia Nacional de Vigilância Sanitária. **RDC nº 107:** Resolução de Diretoria Colegiada de 5 de setembro de 2016. Brasília, 2016. 36 p.

ANVISA, Agencia Nacional de Vigilância Sanitária. **Critérios Diagnósticos de Infecções Relacionadas à Assistência à Saúde**. Brasília, 2017. 90 p.

ARIAS, M. V. B. et al. Estudo da ocorrência de infecção hospitalar em cães e gatos em um centro cirúrgico veterinário universitário. **Pesquisa Veterinária Brasileira**, v. 33, n. 6, p. 771-779, 2013.

AYOUB, F. et al. Chlorhexidine-alcohol versus povidone-iodine for pre-operative skin preparation: A systematic review and meta-analysis. **International Journal of Surgery Open**, v. 1, p. 41-46, 2015.

BELO, L. et al. Skin asepsis protocols as a preventive measure of surgical site infections in dogs: chlorhexidine-alcohol versus povidone-iodine. **BMC Veterinary Research**, v. 14, n. 1, p. 1-6, 2018.

BERALDO, C. C.; ANDRADE, D. Higiene bucal com clorexidina na prevenção de pneumonia associada à ventilação mecânica. **Jornal Brasileiro de Pneumologia**, v. 34, n. 9, p. 707-714, 2008.

BERTOLDO, J. G. et al. Uso ou abuso em testes de comparações de média: conhecimento científico ou empírico? **Ciência Rural**, v. 38, n. 4, p. 1145-1148, 2008.

DIOMEDI, A. et al. Antisépticos y desinfectantes: apuntando al uso racional. Recomendaciones del Comité Consultivo de Infecciones Asociadas a la Atención de Salud, Sociedad Chilena de Infectología. **Revista Chilena de Infectología**, v. 34, n. 2, p. 156-174, 2017.

EDMISTON, C. E. et al. Reducing the risk of surgical site infections: Does chlorhexidine gluconate provide a risk reduction benefit? **American Journal of Infection Control**, v. 41, n. 5, p. 49-55, 2013.

ERCOLE F. F. et al. Risco para infecção de sítio cirúrgico em pacientes submetidos a cirurgias ortopédicas**. Revista Latino-Americana de Enfermagem**, v. 19, n. 6, p. 1362-1368, 2011.

FOSSUM, T. W. **Cirurgia de pequenos animais**. 4. ed. St Louis, MO: MOSBY, 2014. 1195 p.

GARCIA, D. O. et al. Antissepsia cirúrgica oftálmica: Polivilpirrolidona-iodo versus clorexidina. **Investigação**, v. 16, n. 2, p. 1-5, 2017.

GARCIA, H. C. Tabelas para classificação do coeficiente de variação. **IPEF - Instituto de Pesquisas e Estudos Florestais**, n. 171, p. 1-10, 1989.

GOULART, D. R.; ASSIS, E. A.; SOUZA, M. T. Avaliação microbiológica da antissepsia pré-operatória das mãos. **Revista de Cirurgia e Traumatologia Buco-Maxilo-Facial**, v. 11, n. 3, p. 103-112, 2011.

KRUMMENNAUER, E. C.; MENEZES, R. M.; RENNER, J. D. P. Strategies for the prevention and control of surgical infections: from history to the present. **Journal of Infection Control**, v. 8, n. 2, p. 69-76, 2019.

LÓPEZ, A. S. et al. Nanocápsulas de clorexidina na antissepsia cirúrgica de cães. IN: Salão Internacional de Ensino, Pesquisa e Extensão, 8., 2017, Santana do Livramento. **Anais do Oitavo Salão Internacional de Ensino, Pesquisa e Extensão**. Santana do Livramento: UNIPAMPA, 2017. p. 1.

MASUKAWA, I. et al. **POP nº 14 - SCIH/HU:** Procedimento Operacional Padrão (POP) -, de 20 de maio de 2016. 2016. Florianópolis, 2016. 5 p.

MORIYA, T.; MÓDENA, J. L. P. Assepsia e antissepsia: técnicas de esterilização. **Medicina (Ribeirão Preto)**, v. 41, n. 3, p. 265-273, 2008.

OLIVEIRA, A. C.; GAMA, C. S. What to use in preoperative skin preparation: povidone-iodine or chlorhexidine? **Revista SOBECC**, v. 23, n. 3, p. 155-159, 2018.

PRATES, C. G. et al. Comparação das taxas de infecção cirúrgica após implantação do checklist de segurança. **Acta Paulista de Enfermagem**, v. 31, n. 2, p. 116-122, 2018.

REIS, L. M. et al. Avaliação da atividade antimicrobiana de antissépticos e desinfetantes utilizados em um serviço público de saúde. **Revista Brasileira de Enfermagem**, v. 64, n. 5, p. 870-875, 2011.

RODRIGUES, E. A. C. et al. **Infecções hospitalares:** prevenção e controle. 1. ed. São Paulo, SP: SARVIER, 1997. p. 670.

SILVA, D. A. R. et al. O gluconato de clorexidina ou o álcool-iodo-álcool na antissepsia de campos operatórios em cães. **Ciência Rural**, v. 30, n. 3, p. 431-437, 2000.

SILVA, F. M. et al. Eficácia dos antissépticos na antissepsia cirúrgica das mãos. IN: Salão Internacional de Ensino, Pesquisa e Extensão, 7., 2015, Santana do Livramento. **Anais do Sétimo Salão Internacional de Ensino, Pesquisa e Extensão**. Santana do Livramento: UNIPAMPA, 2015. p. 1-2.

URQUIZA, M. C. et al. Comparação da eficácia e efeito residual de duas técnicas de antissepsia pré-operatória das mãos com duas substâncias antissépticas. **Revista Interdisciplinar**, v. 9, n. 3, p. 112-120, 2016.