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Original Research Article

# Effect of four surgical antisepsis protocols on bacteria counts in felines undergoing routine ovariohysterectomy

## *Efecto de cuatro protocolos de antisepsia quirúrgica sobre el recuento de bacterias en felinos sometidos a ovariohisterectomía de rutina*

## *Efeito de quatro protocolos de antissepsia cirúrgica na contagem de bactérias em felinos submetidos a ovariohisterectomia de rotina*

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## Abstract

**Background:** Endogenous microbial flora is the most frequent cause of contamination of the surgical wound and its subsequent infection. Surgical antisepsis is the control of infection in surgical wounds by reducing microbial contamination. **Objective:** The main objective of this research was to determine the effect of four surgical antisepsis protocols on bacterial counts in felines undergoing routine ovariohysterectomy at different moments: Moment 1 ($M_1$) after trichotomy and before antisepsis, Moment 2 ($M_2$) after antisepsis and Moment 3 ($M_3$) at the end of the surgical procedure. **Methods:** Sixty mixed-breed felines, 5 to 12 months of age, were randomly subjected to 4 surgical antisepsis protocols: 7.5% povidone-iodine soap and rinsing with 70° alcohol, 7.5% povidone-iodine soap and rinsing with saline solution, 2% chlorhexidine soap and rinsing with 70°, alcohol and chlorhexidine soap 2% and rinsing with saline solution. **Results:** A numerical reduction in the number of bacteria was observed in all groups. Regarding the comparison of bacterial growth by protocols evaluated, using the Kruskal Wallis test, no statistically significant differences were found between the protocols studied ($p>0.05$). Regarding the comparison of bacterial counts by moments in each protocol (same individuals evaluated at different moments), using the Friedman and Holm test, statistically significant differences ($p<0.05$) were found between the studied moments: $M_1M_2$ $p=4.9^{-11}$; $M_1M_3$ $p=4.9^{-11}$ and $M_2M_3$ $p=0.039$. **Conclusion:** Under the conditions of the present study, any of the four protocols have a similar effect on bacterial reduction in felines undergoing ovariohysterectomy. Regarding the moments studied, there are differences between the 3 moments studied, with $M_1$ being dissimilar to $M_2$, $M_1$ to $M_3$, and $M_2$ to $M_3$.

**Keywords:** *alcohol; bacterial count; cats; chlorhexidine; disinfection; felines; ovariohysterectomy; povidone-iodine; saline solution; surgical antisepsis; surgery.*

## Resumen

**Antecedentes:** La flora microbiana endógena es la causa más frecuente de contaminación de la herida quirúrgica y su posterior infección. La antisepsia quirúrgica es el control de la infección de las heridas quirúrgicas mediante la reducción de la contaminación microbiana. **Objetivo:** El objetivo principal de esta investigación fue determinar el efecto de cuatro protocolos de antisepsia quirúrgica sobre el recuento bacteriano en felinos sometidos a ovariohisterectomía de rutina en diferentes momentos: el Momento 1 ($M_1$) después de la tricotomía y antes de la antisepsia, el Momento 2 ($M_2$) después de la antisepsia, y el Momento 3 ($M_3$) al final del procedimiento quirúrgico. **Métodos**: Sesenta felinos mestizos, de 5 a 12 meses de edad, fueron sometidos aleatoriamente a cuatro protocolos de antisepsia quirúrgica: jabón povidona yodada al 7,5% y aclaramiento con alcohol 70°, jabón povidona yodada al 7,5% y aclaramiento con solución salina, jabón de clorhexidina al 2% y aclaramiento con alcohol 70°, y jabón de clorhexidina al 2% y aclaramiento con solución salina. **Resultados:** Se observó una reducción numérica en el número de bacterias en todos los grupos. En cuanto a la comparación del crecimiento bacteriano por protocolos evaluados, mediante la prueba de Kruskal-Wallis, no se encontraron diferencias estadísticamente significativas entre los protocolos estudiados ($p>0,05$). En cuanto a la comparación del recuento bacteriano por momentos en cada protocolo (mismos individuos evaluados en diferentes momentos), mediante la prueba de Friedman y Holm, se encontraron diferencias estadísticamente significativas ($p<0,05$) entre los momentos estudiados: $M_1M_2$ $p=4,9^{-11}$; $M_1M_3$ $p=4,9^{-11}$, y $M_2M_3$ $p=0,039$. **Conclusiones:** en las condiciones del presente estudio, cualquiera de los cuatro protocolos tiene un efecto similar sobre el protocolo de antisepsia en felinos sometidos a ovariohisterectomía. En cuanto a los momentos estudiados, hay diferencias entre los tres momentos estudiados, siendo $M_1$ diferente a $M_2$, $M_1$ a $M_3$, y $M_2$ a $M_3$.


**Palabras clave:** *alcohol; cirugía; clorhexidina; desinfección; felinos; gatos; ovariohisterectomía; povidona yodada; antisepsia quirúrgica; recuento bacteriano; solución salina.*


## Resumo

**Antecedentes:** A flora microbiana endógena é a causa mais frequente de contaminação da ferida cirúrgica e sua subsequente infecção. A antissepsia cirúrgica é o controle da infecção de feridas cirúrgicas, reduzindo a contaminação microbiana. **Objetivo:** O objetivo principal desta investigação foi determinar o efeito de quatro protocolos de antissepsia cirúrgica na contagem bacteriana em felinos submetidos à histerectomia olivar de rotina em diferentes momentos: o momento 1 ($M_1$) após a tricotomia e antes da antissepsia, o momento 2 ($M_2$) após a antissepsia. e momento 3 ($M_3$) ao final do procedimento cirúrgico. **Métodos:** Sessenta felinos sem raça definida, de 5 a 12 meses de idade, foram submetidos aleatoriamente a quatro protocolos de antissepsia cirúrgica: sabonete de iodeto de povidona 7,5% e enxágue com álcool 70°, sabonete de iodeto de povidona 7,5% e enxágue com álcool 70° e enxaguar com soro fisiológico, sabonete de clorexidina 2% e enxaguar com álcool 70°, e sabonete de clorexidina 2% e enxaguar com soro fisiológico. **Resultados:** observou-se uma redução numérica no número de bactérias em todos os grupos. Quanto à comparação da contagem bacteriana pelos protocolos avaliados, por meio do teste de Kruskal-Wallis, não foram encontradas diferenças estatisticamente significativas entre os protocolos estudados ($p>0,05$). Quanto à comparação do crescimento bacteriano por momentos de cada protocolo (mesmos indivíduos avaliados em momentos diferentes), por meio do teste de Friedman e Holm, foram encontradas diferenças estatisticamente significativas ($p<0,05$) entre os momentos estudados: $M_1M_2$ $p= 4,9^{-11}$; $M_1M_3$ $p=4,9^{-11}$, e $M_2M_3$ $p=0,039$. **Conclusões:** nas condições do presente estudo, qualquer um dos quatro protocolos tem efeito semelhante no protocolo de antissepsia em felinos submetidos à histerectomia olivar. Em relação aos momentos estudados, há diferenças entre os 3 momentos estudados, sendo $M_1$ diferente de $M_2$, $M_1$ de $M_3$, e $M_2$ de $M_3$.


**Palavras-chave:** *álcool; clorexidina; cirurgia; contagem bacteriana; desinfecção; felinos; iodopovidona; ovário-histerectomia; antissepsia cirúrgica; solução salina.*

## Introduction

Surgery poses a risk of infection to patients by disrupting the integrity of the skin, allowing microorganisms to enter. Endogenous microbial flora is the most frequent cause of contamination of surgical wounds, and subsequent infection. (Fossum et al., 2009). The goal of antiseptic techniques is to reduce the level of wound contamination. (Slatter, 2006). For example, iodine can be mixed with polyvinylpyrrolidone, constituting the povidone-iodine complex (iodophors). (Rubio and Boggio, 2009). Iodophors must be in contact with the skin for a minimum of 2 minutes to release a sufficient amount of free iodine to kill bacteria. Their activity is reduced in the presence of organic substances (blood, fat, and necrotic debris) because these compounds convert free iodine into inactive iodine. Alcohol increases the release of free iodine from the iodophors, although alcohol may decrease the persistent action of the iodophor. Iodophors are effective in reducing the number of bacteria on the skin for one hour after application and have persistent activity for 4 to 6 hours, but no residual activity (Slatter, 2006). Chlorhexidine gluconate is insoluble in water, but very soluble in alcohol, making it the most commonly used product in practice. (Sánchez and Sáenz, 2005). Regarding alcohols, their antibacterial action depends on their ability to denature proteins and dissolve lipids. They have variable activity against viruses, with some data indicating that ethanol is more effective than isopropyl alcohol (Botana et al., 2002). The largest number of antisepsis studies related to bacterial load have been conducted in human models, followed by animals, especially canines, but not in felines, where scarce research has been conducted on the use of chlorhexidine.

The objective of this study was to determine the effect of four antisepsis protocols on bacterial count in felines undergoing routine ovariohysterectomy at different time points. We hypothesized that ($H_1$) a 2% Chlorhexidine Gluconate and 70° alcohol rinse would result in a significantly greater reduction in skin bacterial count compared to other protocols, and ($H_2$) bacterial count would differ between time points 2 and 3 relative to time point 1 in the four protocols.

## Materials and Methods

### *Ethical considerations*

This study was approved by the Commission for the Evaluation of Preliminary Projects and Theses of the Faculty of Veterinary Sciences, National University of Asunción, having the informed consent of the guardians of the pets and complying with Law No. 4840 / On Animal Protection and Welfare of the Republic of Paraguay (Legislative Branch of the Republic of Paraguay, 2013).

### *Study design*

This experimental study was conducted in 2023 at VETCIA -a private veterinary center in Asunción-Paraguay. The Laboratory studies were carried out in the Department of Microbiology and Immunology of the Faculty of Veterinary Sciences of the National University of Asunción (San Lorenzo, Paraguay). A total of 60 clinically healthy female cats, aged 5 to 12 months old, classified as ASA I and ASA II were included in the study. All animals were undergoing routine ovariohysterectomy and had measurable microbial load on initial swabbing.

### *Sampling methods*

The type of sampling was multistage. In the first stage, the sampling was non-probabilistic of consecutive cases. In the second stage, sampling was simple random probabilistic, where each feline had the same probability of being part of the study through random selection: Protocol A feline 1, Protocol B feline 2, Protocol C feline 3, Protocol D feline 4, and again Protocol A feline 5, and so on.

### *Sample collection*

For the application of antisepsis protocols, A, B, C and D of the operating field, these cases were distributed randomly, while sampling from the skin surface once the protocols were

applied and the surgical technique started, was carried out double blind, where the operator who took the samples during surgery from the skin surface, and the operator who carried out the microbiological cultures from said samples, were unaware of the antiseptic technique applied to them.

### *Antisepsis protocol*

The trichotomy was performed using an electric razor, once the patient was pre-medicated and induced into surgical anesthesia. The first sample ($M_1$) was obtained by means of a skin swab at the site where the skin incision would be made (middle part of the abdominal region) by gently rubbing the surface in 10 movements, in the direction of the long axis of the incision. The sample was identified by patient and sample number, kept refrigerated in Stuart transport until sent to the Laboratory, in a period of no more than 24 hours. Then, the random distribution of each feline patient with microbial load to one of the four antisepsis protocols evaluated was carried out as can be seen in Table 1, having a total of 15 animals for each protocol.

**Table 1.** Protocols of antisepsis in different groups.

| Protocol | Washes | Rinse | Embrocation |
|---|---|---|---|
| **A** | 3 washes with 7,5 % povidone-iodine soap | 70 ° alcohol | 1 % povidone-iodine solution |
| **B** | 3 washes with 7,5 % povidone-iodine soap | Saline solution | 1 % povidone-iodine solution |
| **C** | 3 washes with 2 % chlorhexidine soap | 70 ° alcohol | 0.5% chlorhexidine solution |
| **D** | 3 washes with 2 % chlorhexidine soap | Saline solution | 0.5% chlorhexidine solution |

The skin contact time with the antiseptic agent was at least 2 minutes for all antiseptics used in the study. The swabs (sterile) were handled with gloved hands using an aseptic technique. The dominant hand grabbed the swab to perform the sterile preparation, while the non-dominant hand was used to retrieve the swabs from the preparation container. The washing of the skin surface began at the incision site, near the center of the shaved area, through a circular motion from the center to the periphery. After the embrocation, and limited to the operating field, the second sampling ($M_2$) of the skin surface was carried out. The third sample ($M_3$) was obtained at the end of the surgical procedure in the lateral area of the sutured surgical wound.

### *Determination of bacterial count*

The samples were processed by the mesophile counting technique to obtain the number of bacteria in colony-forming units (CFU). The swab containing the sample was placed in a tube with 9 ml of peptone water. From here, successive dilutions were made by removing 1 ml from the tube and placing it in 9 ml of peptone water, this was carried out until the $10^{-6}$ dilution was obtained. Once the dilutions were made, they were cultured in duplicate on count agar plates, placing 100 µl on each plate. All plates were placed in the oven at 37 °C. Then, after 24 hours of incubation, counting was carried out. Those plates containing 30 to 300 colonies were counted and multiplied by the inverse of the dilution factor.

### *Statistical analysis*

The results of the effect of four antisepsis protocols were measured quantitatively according to colony-forming units (CFU) per milliliters. To determine if the differences

observed were statistically significant or due to chance, several statistical tests were used. The Shapiro-Wilk test was used to verify normality, and the Fligner-Killeen test was used to verify homoscedasticity. The Kruskal- Wallis test was applied to compare the protocols at each evaluation point, while the Friedman test was used to compare bacterial count at the three different times, as it involved evaluating the same individuals at different times. For post hoc comparisons, the Holm method was used. Analyses were performed using R software (R Core Team, 2023). In all tests the p value of $p< 0.05$ was considered significant.

## Results

Table 2 shows the results of the descriptive statistics in CFU/ml can be seen. The values of the standard deviation and the range, especially at evaluation $M_1$, indicate a marked dispersion of the observations; likewise, in the four protocols studied, higher average and median values were observed at $M_1$ after antisepsis and embrocation, However, at $M_2$, a drastic reduction in both resident and transient microorganisms on the skin of the felines under study was observed. Specifically, a median of 0.0001 CFU/ml was observed across all protocols. At the end of the surgical procedure ($M_3$), the same median value of 0.0001 CFU/ml was observed in all four protocols.

**Table 2.** Descriptive statistics of bacterial count expressed in CFU/ml of the study protocols.

| Antiseptic | Time | Mean | Median | D.E. | Min | Max | Range |
|---|---|---|---|---|---|---|---|
| *Protocol A* | $M_1$ | 1227 | 60 | 3010 | 10 | 10000 | 9990 |
| | $M_2$ | 0.0001 | 0.0001 | 0 | 0.0001 | 0.0001 | 0 |
| **IOP and alcohol** | $M_3$ | 3.33 | 0.0001 | 8.16 | 0.0001 | 30 | 29.9999 |
| *Protocol B* | $M_1$ | 3091 | 900 | 5761 | 20 | 20000 | 19980 |
| | $M_2$ | 4.67 | 0.0001 | 10.6 | 0.0001 | 30 | 29.9999 |
| **IOP and saline solution** | $M_3$ | 4 | 0.0001 | 9.10 | 0.0001 | 30 | 29.9999 |
| *Protocol C* | $M_1$ | 4990 | 200 | 17992 | 10 | 70000 | 69990 |
| | $M_2$ | 0.0001 | 0.0001 | 0 | 0.0001 | 0.0001 | 0 |
| **Chlorhexidine and alcohol** | $M_3$ | 2 | 0.0001 | 7.75 | 0.0001 | 30 | 29.9999 |
| *Protocol D* | $M_1$ | 7043 | 1000 | 20348 | 10 | 80000 | 79990 |
| | $M_2$ | 1.33 | 0.0001 | 3.52 | 0.0001 | 10 | 9.9999 |
| **Chlorhexidine and saline solution** | $M_3$ | 75.3 | 0.0001 | 257 | 0.0001 | 1000 | 999.999 |

To improve the symmetry of the distribution and optimize its graphical visualization, the statistical data were transformed to a logarithmic base of 10. Two tests were carried out to establish the use of parametric or non-parametric tools. In this regard, Table 3 shows the Normality Test using the Shapiro-Wilk Test. At all times, a $p<0.05$ was observed, which demonstrated a distribution not adjusted to normal.

**Table 3.** Normality test using the Shapiro Wilk test for bacterial count of the study protocols.

| Moment | Statistical | N | p-value |
|---|---|---|---|
| **M1** | 0.94751 | 60 | 0.01191 |
| **M2** | 0.31354 | 60 | $3.06e^{-15}$ |
| **M3** | 0.51575 | 60 | $8.99e^{-13}$ |

Table 4 shows the results of the homoscedasticity test using the Fligner-Killeen test with a logarithmic base of 10. A p-value greater than 0.05 was obtained, indicating homogeneity of variance.

**Table 4.** Homoscedasticity Test (homogeneity of variances) using the Fligner-Killeen test for bacterial count of the study protocols.

| Moment | Statistical | N | P-value |
|---|---|---|---|
| **M1** | 1.127 | 60 | 0.7706 |
| **M2** | 6.1499 | 60 | 0.1045 |
| **M3** | 3.960 | 60 | 0.2648 |

Based on these data and considering both the distribution and homogeneity of variances, the non-parametric Kruskal-Wallis test was selected for the inferential analysis to compare bacterial count between protocols at each of the evaluation moments (M1, M2, and M3).

**Table 5.** Kruskal Wallis test (comparison of bacterial count per protocol evaluated at each time point).

| | Median | | | |
|---|---|---|---|---|
| | ***Protocol*** | ***CFU/ml*** | ***Log. 10*** | ***P-value*** |
| **M1** | A | 60 | 1.78 | 0,0539695 |
| | B | 900 | 2.95 | |
| | C | 200 | 2.30 | |
| | D | 1000 | 3 | |
| | ***Protocol*** | ***CFU/ml*** | ***Log. 10*** | ***P-value*** |
| **M2** | A | 0.0001 | -4 | 0,1163731 |
| | B | 0.0001 | -4 | |
| | C | 0.0001 | -4 | |
| | D | 0.0001 | -4 | |
| | ***Protocol*** | ***CFU/ml*** | ***Log. 10*** | ***P-value*** |
| **M3** | A | 0.0001 | -4 | 0,3316285 |
| | B | 0.0001 | -4 | |
| | C | 0.0001 | -4 | |
| | D | 0.0001 | -4 | |

In Table 5, the probability value was greater than 0.05 ($p > \alpha$) when comparing bacterial count with logarithmic transformation based on 10 per protocol, evaluated at each time point. This indicates statistical similarity, eliminating the need for additional post hoc tests. Likewise, in order to compare bacterial count in the same individuals at different times with each protocol, paired or dependent data were used, and the non-parametric Friedman test was selected as a statistical tool.

**Table 6.** Friedman test (comparison of bacterial counts by moments in each protocol – same individuals evaluated at different moments) and Holm Test for differences between moments.

| **Friedman test (comparison of bacterial growth by moments in each protocol)** | **P-value** $< 2{,}2e^{-16}$ |
|---|---|
| **Holm Test** | |
| **Contrast** | **p-value** |
| **$M_1$ vs. $M_2$** | $4.9e^{-11}$ |
| **$M_1$ vs. $M_3$** | $4.9e^{-11}$ |
| **$M_2$ vs. $M_3$** | 0.039 |

Table 6 summarizes the results of the Friedman test, which assessed differences in bacterial counts across time points within the same individuals. There were statistically significant differences ($p<0.05$) between time points, regardless of the antisepsis protocol used. Post hoc comparisons using the Holm Test further confirmed significant differences between all three time points ($M_1$ vs. $M_2$, $M_1$ vs. $M_3$, and $M_2$ vs. $M_3$). These findings suggest a progressive reduction in bacterial counts following antisepsis and throughout the surgical procedure.

## Discussion

All four antisepsis protocols significantly reduced the bacterial load on the skin of felines undergoing routine ovariohysterectomy, with the most notable reduction observed at $M_2$. This reduction was maintained at $M_3$. This reduction across all protocols suggests their effectiveness in minimizing the risk of surgical site infections, with slight variations in the effectiveness of different protocols, which warrants further investigation. Statistical analyses confirmed the homogeneity of variance and the absence of significant differences between protocols at each evaluation point. The use of the Friedman test for paired data provided robust comparisons of bacterial growth at different times, reinforcing the reliability of the findings.

Considering the antiseptics used, it is necessary to mention that Chlorhexidine Gluconate has a slower activity than alcohols. Its effectiveness has been proven to reduce the skin flora after a few seconds of application. Chlorhexidine has good activity against gram-negative bacteria, but less so against gram-negative bacteria and fungi. It has a residual action of more than 6 hours. It is considered the "Gold standard" of antiseptics. Very few animal studies have documented the cutaneous pharmacokinetics of chlorhexidine, although several human studies have shown that chlorhexidine is not easily absorbed through the skin. Iodophor formulations used as antiseptics contain less free iodine than those formulated as disinfectants (between 0.75 – 1.2% available iodine). Their residual activity is minimal, lower than other antiseptics, and their antimicrobial activity is neutralized by organic matter. (Hernández and Negro, 2013).

Iodophors have been shown to have a broad spectrum of activity against some microbes, such as vegetative bacteria, viruses, fungi, mycobacterium, and protozoa (Botana et al., 2002). Iodophors must be in contact with the skin for

a minimum of 2 minutes to release a sufficient amount of free iodine to kill bacteria. Alcohol increases the release of free iodine from iodophors, so these products are often used together, although alcohol may decrease the persistent action of the iodophor. After scrubbing, free iodine diffuses into deeper regions of the skin, providing some degree of persistent action (Slatter, 2006).

Regarding the bacterial load necessary to produce an infection, Hernández and Negro (2013) mention that said load must be equal to or greater than $10^5$ CFU/ml, implying a very high risk of infection. They establish that the presence of bacteria is not the only condition for wound infection to develop; the state of the patient's defense mechanisms must be considered, as well as the external factors of the patient's prolonged stay in the hospital before surgery or the duration of surgery. In the present study, loads equal to or greater than that mentioned by Hernández and Negro were obtained at $M_1$, which could predispose wounds to infection. However, the antiseptics studied drastically decreased this load after their application, highlighting the importance of antisepsis in surgery. It should also be mentioned that Botana et al. (2002) describe that dilution in saline serum can cause its precipitation and alteration of pH, but based on the results obtained in this study, and under its conditions, that phenomenon was not observed due to the drastic decrease in counts. Sumano and Ocampo (1997) mention that chlorhexidine is compatible with other cationic substances such as quaternary ammonium compounds. While it is true that virulence and bacteria count are important, the inoculum is no less. Thus, bacteria lodged in a multifilament suture material greatly increase the risk of infection, with $10^2$ CFU/g being enough to develop it. Every time an incision is made in tissue, the wound will be contaminated with germs from the body itself, as well as, to a lesser extent, from the environment (Hernández and Negro, 2013).

Gibson et al. (1997) evaluated a one-step iodophor skin preparation solution (iodophor with 0.7% available iodine in isopropyl alcohol) and the application of chlorhexidine gluconate as a skin preparation method in canines and felines undergoing elective ovariohysterectomy. Preoperative and intraoperative skin cultures demonstrated no differences in antiseptic efficacy. The results of the present study agree with Gibson et al. (1997), since no statistical differences were found between the study groups, nor were there alterations in healing. Assuming that the microbial flora of the skin in canines and felines could be considered different, this work was considered similar since they are the most frequently intervened domestic species in the daily clinic. Belo et al. (2018) evaluated the effectiveness of two pre-surgical skin asepsis protocols in 46 dogs which were randomly assigned to an antisepsis protocol with a 7.5% aqueous povidone-iodine solution or with an alcoholic chlorhexidine solution at 2%. The results showed that the majority of the samples collected post-asepsis did not present bacterial growth, both for the animals subjected to the povidone-iodine (74%) or chlorhexidine (70%) protocols. In only 9% of cases was no significant log reduction observed, indicating possible resistance to these agents. Furthermore, the logarithmic reduction of the bacterial quantification of the pre- and post-aseptic time was not statistically different for povidone-iodine (6.51 protocol ± 1.94 log10) and chlorhexidine (6.46 ± 2.62 log10). The data presented by Belo et al. (2018) coincide with the results of the present study, since logarithmic regression was also applied within the design; Although it is a different species, the result is applicable to surgical asepsis in general. (Scott et al., 2002; Abraham et al., 2007).

Marroquin in 2008; determined the effectiveness of 0.5% chlorhexidine diacetate as a pre-surgical skin antiseptic. Pre-surgical skin preparation was performed on 15 canine patients using 0.5% chlorhexidine diacetate as an antiseptic and in another 15 canine patients using 15% povidone iodine as an antiseptic. The study demonstrated the immediate and residual effectiveness of 0.5% chlorhexidine diacetate as a pre-surgical skin antiseptic. Although both agents had an acceptable antiseptic response, 0.5% chlorhexidine diacetate was more economical.

Boucher et al. (2018) compared the antimicrobial efficacy of a 2% chlorhexidine gluconate and 70% ethanol solution (CG1A) with that of F10 Skin Prep Solution® (F10) and electrochemically activated water (EOA) when used as surgical preparation in canine patients. Boucher's results partially coincide with those reported in the present study in terms of the study moments, but they only found differences in the first sampling time, not in the three moments as in the present study, probably due to the characteristics of the products used.

Considering the hypotheses raised $H_1$: In the antisepsis of the surgical field, 2% Chlorhexidine Gluconate and 70° alcohol rinse have a greater reduction effect on the number of bacteria on the skin of felines undergoing routine ovariohysterectomy, at different times, in relation to the other protocols; it is not supported, because no statistically significant differences ($p>0.05$) were found between the 4 protocols evaluated; in other words, under the conditions of the present study, any of the 4 protocols have a similar effect on the antisepsis protocol in felines undergoing ovariohysterectomy; and $H_2$: In antisepsis of the surgical field, the number of bacteria on the skin in felines undergoing routine ovariohysterectomy is different at moments 2 and 3, in relation to moment 1 in the four protocols; it is supported because, regarding the moments studied, according to the Friedman Test and the Holm Test, there were statistical differences ($p < 0.05$) between the 3 moments studied: $M_1M_2$ $p=4.9^{-11}$; $M_1M_3$ $p=4.9^{-11}$, and $M_2M_3$ $p=0.039$.

## Declarations

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### *Conflicts of interest*

The authors declare they have no conflicts of interest regarding the work presented in this report.

### *Author contributions*

RGV: Investigation, project administration, and methodology. CEBV: Methodology in experimental phase. MIRA and YJBM: Data analysis. ELMA: Methodology. MBLN: Methodology and Writing – Original Draft Preparation. LCCB: Microbiological analysis. XCP: Methodology and Writing – Original Draft Preparation.

### *Use of artificial intelligence (AI)*

No AI or AI-assisted technologies were used during the preparation of this work.

### *Data availability*

The data sets used in the current study are available from the corresponding author on request.

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