Preoperative skin asepsis protocols using chlorhexidine versus povidone-iodine in veterinary surgery: A systematic review and meta-analysis

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Abstract
Objective: To provide a systematic assessment of the efficacy of preoperative skin asepsis using chlorhexidine versus povidone-iodine based protocols for surgical site infection (SSI) prevention in veterinary surgery.
Study design: Systematic meta-analytical review according to PRISMA-P guidelines.
Sample population: Studies comparing preoperative skin asepsis protocols using chlorhexidine versus povidone-iodine in veterinary surgery identified by systematic search between 1990 and 2020.
Methods: A search using MEDLINE/Pubmed, Web of Science and CAB Abstracts was performed, followed by secondary searches of Google Scholar, Proquest Dissertation and Theses, and relevant bibliographic articles. Primary and secondary outcome measures were the efficacy of skin asepsis protocols using chlorhexidine versus povidone-iodine on SSI incidence and skin bacterial colonization, respectively. A meta-analysis was performed with a random-effect model, with effect size calculated as risk ratio (RR) or mean standard deviation (MSD) with 95% CI. Statistical significance was set at $P < .05$.
Results: Among 1067 publications that met the initial search criteria, 9 relevant studies were eligible for analysis. No difference in the incidence of postoperative SSI or skin bacterial colonization between preoperative asepsis protocols using chlorhexidine versus povidone-iodine was found. Insufficient information and detail were frequent among studies and precluded a clear assessment of bias.
Conclusion: This study showed that asepsis protocols using chlorhexidine were comparable to povidone-iodine in preventing postoperative SSI and reducing skin bacterial colonization.
Clinical significance: Given the limitations of the studies that were included in terms of both quality and quantity, more high-quality randomized controlled trials are needed to confirm these conclusions.

1 | INTRODUCTION

Postoperative surgical site infection (SSI) is a leading cause of morbidity and mortality and represents a major economic and welfare burden in veterinary surgery.1–3 Preoperative surgical site asepsis practice is based on the knowledge that pathogens leading to SSI are often acquired from the patient’s endogenous flora. Hence, preoperative surgical site skin asepsis using appropriate products represents one of the most critical factors for SSI prevention. Aseptic protocols in veterinary surgery are traditionally based on methods used in humans. They typically involve the use of an aqueous or alcohol-based preparation of chlorhexidine or povidone-iodine. Despite chlorhexidine’s wider range of antimicrobial activity compared to povidone-iodine, concerns may arise with its use, as methicillin-resistant Staphylococcus aureus (MRSA) and enterococci may develop acquired resistance to it.4,5 Moreover, a longer residual effect and a greater skin tolerance have historically been attributed to chlorhexidine, characteristics that may have been overestimated.6–9 Multiple veterinary studies have comparatively investigated SSI occurrence and/or bacterial reduction at the surgical site using chlorhexidine and povidone-iodine based protocols but were unable to draw conclusions about which surgical site antiseptic should be preferred.10–17 According to recent systematic reviews in human patients, chlorhexidine appears superior to povidone-iodine in preventing SSI, with up to 30% reduction in its incidence, and in reducing skin bacterial colonization.18,19 Nevertheless, criticism has arisen regarding metanlyses promoting the use of chlorhexidine-alcohol over povidone-iodine with or without alcohol in preventing SSI because of inclusion of studies with unknown active ingredient concentrations or concentrations below the active range.20

Despite some evidence in favor of chlorhexidine, this may not apply to veterinary surgery where patients present with different skin characteristics and microflora than human patients. As the choice of antiseptic should be based on the best available empirical data, including efficacy, patient tolerance and resistance development, or its potential, the objective of this study was to provide a systematic meta-analytic assessment of the efficacy of preoperative skin asepsis protocols using chlorhexidine versus povidone-iodine for SSI prevention and skin bacterial burden reduction in veterinary surgery. Considering the relationship between skin bacterial flora and SSI, and the aim of skin asepsis being reduction of skin flora, the evaluation of the reduction of both is necessary. We therefore, hypothesized that preoperative skin preparation with chlorhexidine-based protocols would be superior to that of povidone-iodine in both prevention of SSI and reduction of skin bacterial colonization.

2 | MATERIALS AND METHODS

This review was conducted according to the recommendations in the PRISMA-P 2015 statement.21

2.1 | Literature search strategy

A comprehensive literature search was carried out on February 4, 2021. The MEDLINE/Pubmed, Web of Science Core Collection and CAB Abstracts databases were searched, with University of Bern institutional access, for published veterinary literature comparing chlorhexidine and povidone-iodine based protocols for preoperative skin asepsis. Additional searches were performed for gray literature with Google Scholar, Proquest Dissertation and Theses Global, and hand searching of reference lists and book chapters. No language restrictions were applied. Retrieval strategy was a combination of keywords, free words, and subject words to include multiple animal species, methods, and end-points. The complete search strategy used can be found in the supplementary material S1. The reference management software EndNote X9 (Clarivate, Philadelphia, Pennsylvania) was used to import references from searched databases, remove duplicate references, screen by title and abstract, retrieve and screen full text documents, and record reasons for exclusions.

2.2 | Selection criteria

Two reviewers (EM and CC) individually assessed studies to determine eligibility. Disagreements between reviewers were solved by consensus or by the decision of a third reviewer (AS) if consensus between the 2 main reviewers was not achieved. Articles were included if meeting the following criteria: (1) they were randomized controlled trials (RCTs) or observational studies on veterinary patients;
(2) they compared chlorhexidine-based and povidone-
iodine-based preoperative skin asepsis protocols; (3) they
assessed surgical patients; and (4) they assessed at least
1 of the outcomes of interest, being incidence of SSI or
skin bacterial colonization. The latter was defined as a
dichotomous variable being presence or absence of coloni-
zation, or as a continuous variable being the percentage of
bacterial reduction. Human, noncomparative, in vitro or
ex vivo studies, as well as reviews and meta-analyses were
excluded. Further exclusion criteria included studies
where relevant data could not be obtained from the publi-
ished results and the raw data was not made available after
contact with the corresponding authors. The primary and
secondary outcome measures for this study were the effi-
cacy of skin asepsis protocols using chlorhexidine versus
povidone-iodine on the incidence of SSI and skin bacterial
colonization respectively.

2.3  Data collection and analysis methods

Data were collated in Excel (Microsoft, Redmond,
Washington) and included authors’ names, journal name,
article title, year of publication, type of study, species,
type of surgery, sample size of groups, type of skin aseptic
protocols (formulations and application method), SSI
rates, immediate and delayed bacterial colonization. When
needed, missing information and clarification about the statistics presented was sought from the
authors or was obtained using direct algebraic relationships with the available measures of variation. Briefly, when not available, standard deviation of each group was obtained using the standard error of the mean and multiplying it by the square root of the sample size (Eq. 1) or by dividing the width of the 95% confidence interval (known to be 3.92 standard error wide; 3.92=2×1.96) by 3.92, and then multiplying it by the square root of the sample size (Eq. 2).

Eq. 1: Equation to obtain standard deviations from
standard errors for group means

\[ SD = SE \times \sqrt{N} \]  

(1)

Eq. 2: Equation to obtain standard deviations from con-
idence intervals for group means

\[ SD = \sqrt{N} \times (\text{upperlimit}-\text{lowerlimit})/3.92 \]  

(2)

For studies with only summary statistics calculated after
a log-transformation has been applied to the raw data, statistical measures were obtained by finding the means and confidence intervals of the natural logs and taking
their exponentials (antilogs). Separate statistical ana-
lyses using a random-effect model were performed using
Revman 5.4 software (Review Manager, The Cochrane
Collaboration 2020, Oxford, UK) to determine the risk
together (RR) with 95% CI as the effect measure for dichoto-
mous variables and the standard mean difference (SMD)
with 95% CI as the effect measure for continuous data.
The heterogeneity between the studies was analyzed
using the \( \chi^2 \) test, and the size of heterogeneity was
quantified with \( I^2 \), with values of 25%, 50%, and 75% rep-
resenting small, moderate and high heterogeneity. The
significance of the \( I^2 \) value was determined after evalua-
tion of magnitude and direction of effect and strength of
evidence for heterogeneity (\( P \) value of the \( \chi^2 \) test). The
quality of RCTs was assessed using the Cochrane risk of
bias tool, which includes random sequence generation,
allocation concealment, performance bias, detection
bias, attrition bias, reporting bias, and other sources of
bias.

3  RESULTS

3.1  Quantity of research available

The literature search yielded 1291 results through data-
base searching; 4 additional records were identified
through other sources. After removing duplicates, 1067
articles were retained. After screening titles and abstracts,
30 potentially relevant articles were retained for full text
review. Twenty-one studies did not meet the inclusion
criteria (Figure 1). A total of 9 RCTs were judged eligible
and were included in this review. 

3.2  Study characteristics

An overview of the included studies is provided in
Table S2 in the supplementary material. The articles
selected for inclusion were published between 1990 and
2020. The species on which aseptic protocols effi-
cacy was studied were small animals (4 studies, 446 ani-
mals), cattle (2, 164), laboratory mice (2, 72), and
horses (1, 36). Surgery types included in the studies
were classified as clean in 7 studies,\( 11,14,16,24,25 \) and as
mixed clean and clean contaminated in 2.\( 11,13 \) One
study reported SSI incidence as a measure of aseptic
protocol efficacy and did not report skin bacterial colo-
nization.\( 12 \) Three studies reported both SSI and skin bac-
terial colonization,\( 13,15,17 \) whereas 5 reported skin
bacterial colonization only.\( 11,14,16,24,25 \) Diagnosis of SSI
was made according to the Centers for Disease Control
and Prevention (CDC) criteria in 2 studies;\( 13,17 \) in the
others, assessment was based on predefined clinical criteria.\textsuperscript{12,15} The follow-up period for detection of SSI was 30 days in 3 studies\textsuperscript{13,15,17} and limited to 14 days in 1 study.\textsuperscript{12} Immediate skin bacterial colonization was measured at the preoperative timepoint in all studies, and delayed bacterial colonization was measured at the end-of-surgery timepoint in 5 studies\textsuperscript{11,13,15,24,25} or at a predefined time (60, 180 min) in 2 studies.\textsuperscript{14,16}

Formulation, concentration, and application method of antiseptics were not consistent across studies. Four studies reported use of 4\% chlorhexidine gluconate,\textsuperscript{11,13,15,16} 3 studies of 2\% chlorhexidine gluconate,\textsuperscript{14,17,24} and 1 study of 0.5\% chlorhexidine acetate\textsuperscript{12} in alternating order with alcohol and/or saline solution. In 1 study, 2 formulations of chlorhexidine were used, being either 4\% gluconate or a hydro-alcoholic preparation (2\% chlorhexidine gluconate/70\% isopropyl alcohol) depending on the subgroup.\textsuperscript{25} Four studies reported povidone-iodine formulations with a concentration of free iodine of 0.75\%,\textsuperscript{11,13,16,17} and 3 studies of 1\%,\textsuperscript{12,15,24} they were used alone or alternated with alcohol or saline solution. One study used povidone-iodine as a hydroalcoholic preparation (0.7\% iodine/74\% isopropyl alcohol)\textsuperscript{14} and another study either as 1\% or as a hydroalcoholic solution depending on the subgroup.\textsuperscript{25} When alcohol was alternated with chlorhexidine or povidone-iodine, it was 70\% isopropanol in 7 studies\textsuperscript{11,13–17,25} and 70\% ethanol in one study.\textsuperscript{24}

3.3 | Surgical site infection

One study reported no SSI occurrence in either group\textsuperscript{17} and the remaining 3 found no significant statistical difference between the SSI rates for the 2 aseptic protocols.\textsuperscript{12,13,15} Meta-analysis indicated no difference in the incidence of postoperative SSI between preoperative asepsis protocols using chlorhexidine versus povidone-iodine (RR, 0.90; 95\% CI, 0.38-2.12; \(P = 0.82, I^2 = 0\%\); Figure 2).

3.4 | Skin bacterial colonization

Skin bacterial colonization was reported as presence or absence of >5 colony-forming units (CFU) in the immediate and delayed period in 2 studies,\textsuperscript{14,25} or as immediate and delayed reduction factors in 2 studies,\textsuperscript{13,15} in 4 studies both measures were reported.\textsuperscript{11,14,16,24} All studies reported no difference in skin bacterial colonization. Two studies, however, reported a trend toward a reduction in skin bacterial colonization with the use of chlorhexidine and alcohol,\textsuperscript{13,24} while the use of chlorhexidine and saline or povidone-iodine and alcohol performed slightly better than chlorhexidine and alcohol in 2 other studies.\textsuperscript{11,16} Of the 8 studies that evaluated skin bacterial colonization, only 4 used neutralizing agents in sampling.\textsuperscript{11,13,15,16}
Meta-analyses showed no difference for the presence/absence of skin bacterial colonization at immediate (RR, 1.06; 95% CI, 0.52-2.15; P = 0.88, I^2 = 0%; Figure 3) or delayed timepoints (RR, 1.53; 95% CI, 0.38-6.22; P = 0.55, I^2 = 56%; Figure 4), nor for the immediate (SMD, −0.16; 95% CI, −0.41-0.09; P = 0.22, I^2 = 0%; Figure 5) or delayed (SMD, 0.05; 95% CI, −0.20-0.29; P = 0.72, I^2 = 0%; Figure 6) percentage of skin bacterial reduction.
3.5 Risk of bias assessment

The proportions of the studies with low, high, and unclear risks of bias in each domain based on reviewers’ judgment is depicted in Figure 7, and the risk of bias judgment of each study in each domain is illustrated in Figure 8. There was an unclear risk of bias due to insufficient information and detail provided.
in the articles in 2 or more domains in all the included studies. Randomization was judged to have an unclear risk of bias in all the studies as the authors did not clearly state the method of random sequence generation. For allocation concealment, 2 studies had a high risk of bias as they allocated animals either using an alternate sequence or based on the hospital registration number.13,14 Three studies clearly stated the blinding of personnel and outcome assessors,11,12,16 while the other studies did not specify. However, blinding is considered unlikely in these studies as they compare skin antiseptics with different appearance and clinical evaluation of SSI might have been affected by lack of blinding. All studies were judged to have low risk of attrition bias as loss of subjects from study groups was <20% and, when present, equal between groups.

**FIGURE 8** Risk of bias summary: reviewers’ judgment about each risk of bias item for each study that was included

4 | DISCUSSION

The occurrence of SSI is multifactorial. Skin preparation and surgical site bacterial colonization are factors to consider.26 Human studies have provided some evidence supporting the use of chlorhexidine for preoperative skin asepsis and its association with lower bacterial colonization when compared to povidone-iodine.18,19 This is the first systematic meta-analytical review comparing preoperative asepsis protocols using chlorhexidine versus povidone-iodine in veterinary surgery. The results of the current study, however, were not able to provide evidence that chlorhexidine-based protocols were superior to povidone-iodine-based protocols in reducing both rates of SSI and bacterial colonization in veterinary surgery. Clinicians may, therefore, consider other characteristics such as costs, potential side effects for both patients and health care professionals, and potential antiseptic resistance development when choosing the most appropriate preoperative aseptic protocol.

The overall quality of evidence provided by the eligible studies contained within this systematic review was low, which could partially be due to the small number of studies included in each comparison. The variable risk of bias of the included studies could also have affected our ability to estimate effects. Moreover, lack of power in the analysis, as evidenced by the small number of studies and the small sample sizes in each comparison, may affect the confidence in interpreting the results because of the inability to determine whether results that are not statistically significant are indicative of true effects or merely of insufficient data for detecting differences.

Multiple other limitations related to the studies that were included must also be considered when interpreting the meta-analysis results. Variations in the concentration, formulation and application method of the antiseptics constitute a source of clinical heterogeneity in both the individual study protocols and the meta-analysis itself, which makes it difficult to draw strong conclusions. Many antiseptics can be used singly or in combination in a variety of products that may vary considerably in efficacy. This review was intended to compare chlorhexidine- and povidone-iodine-based asepsis protocols rather than individual antiseptics. Results regarding immediate effect of asepsis protocols should therefore be interpreted carefully when alcohols are used in association with chlorhexidine and povidone-iodine to prevent overestimation of the efficacy of the latter.7 Evidence on delayed efficacy of chlorhexidine and povidone-iodine asepsis protocols may reflect more reliably the true effect of the individual antiseptic because, even if used in combination with alcohols, these latter do not show a sustained effect. When interpreting data on SSI occurrence in correlation with antiseptic use, one must
consider that multiple factors, patient related and not patient related, may influence the potential development of SSI, and therefore draw careful conclusions. Moreover, absence of neutralizing agents in sampling media may foster misinterpretation of results as residual bacteriostatic or bactericidal activity may persist therefore rendering impossible to distinguish whether low/absent skin bacterial colonization was obtained before or after sampling. 8

Likewise, although all studies included were RCTs, the differences in methodology are another source of variability that can affect the meta-analysis. Although the evidence gathered in this review does not suggest that asepsis protocols using chlorhexidine are superior to povidone-iodine, it highlights the paucity of the available research, and further trials in preoperative skin asepsis are warranted. Future studies should be planned as rigorous RCTs to reduce bias by ensuring presence of comparable groups through appropriate randomization methods and allocation concealment, as well as by guaranteeing blinding of personnel and outcome accessors. The inclusion of primary research into meta-analyses strongly relies on systematic, appropriate, and transparent reporting of data and detailed description of methodology used. Therefore, high quality standards and compliance with guidelines describing study design, sample size determination, methods of randomization and blinding, will increase the soundness and quality of future meta-analyses.

Studies of skin asepsis tend to use variable endpoints, with most of the studies that were included relying on quantitative skin cultures to determine the antiseptic efficacy as the aim of skin asepsis is to reduce skin flora. However, these outcomes are difficult to correlate with clinical infection, rendering comparison of the results difficult. Postoperative SSI is a clinical diagnosis and is dependent on the recorder. The lack of appropriate and uniform SSI definitions could again make the results difficult to compare. In addition, a large number of SSI could be underreported if superficial and mild, and diagnosed and treated by referring veterinarians after hospital discharge. In fact, a prospective SSI surveillance study in dogs has shown that only 65% of SSI are reported in the medical record, which could have reduced the chances to detect a significant difference between the aseptic protocols. 27

Because of the comprehensive search strategy employed in this review, it is unlikely that eligible published studies were missed. However, there may be relevant unpublished research that was not included. Screening of studies, data extraction and bias assessment were carried out by 2 review authors, limiting potential bias in the review process.

In conclusion, this study showed that preoperative asepsis protocols using chlorhexidine appear to be comparable to povidone-iodine in preventing postoperative SSI and in reducing bacterial colonization in veterinary surgery. Given the limitations of included studies in quality and quantity, more high-quality RCTs with larger sample sizes are needed to further confirm or reject these conclusions.

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CONFLICT OF INTEREST
The authors declare no conflicts of interest related to this report.

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