Original Research Article

Benzalkonium Chloride as a Potential Oral Antiseptic in Veterinary Dentistry: An In Vitro Pilot Study

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ABSTRACT

Benzalkonium chloride (BAC) is a widely used quaternary ammonium compound with broad-spectrum antimicrobial properties; however, its effectiveness against veterinary oral bacteria remains underexplored. This study evaluated the antibacterial activity of BAC at concentrations of 0.1%, 0.5%, 1%, 3%, and 5% using disk diffusion assays on bacterial isolates obtained from a feline oral sample. A descriptive trend of increasing inhibition zone diameter was observed with increasing concentration, ranging from 12 mm at 0.1% to 29 mm at 5%. These findings suggest concentration-dependent antibacterial activity within the tested range. However, due to methodological limitations, including a lack of bacterial identification, the absence of statistical testing, and the use of a single biological sample, results should be interpreted cautiously. This study provides preliminary evidence supporting BAC's potential as an oral antiseptic in veterinary contexts and highlights the need for further controlled investigations.

Keywords: benzalkonium chloride; antibacterial activity; oral bacteria; quaternary ammonium compounds; veterinary dentistry

INTRODUCTION

Benzalkonium Chloride (BAC) is a quaternary ammonium compound widely used as a disinfectant and antiseptic. First introduced in 1935 and later registered with the U.S. Environmental Protection Agency, it is found in cleaning products, cosmetics, agricultural solutions, and medical preparations (1). BAC exerts broad-spectrum antimicrobial activity by disrupting bacterial membranes and denaturing proteins (2).

In humans, BAC is used in pharmaceuticals, mouthwashes, and topical disinfectants, and has been explored for therapeutic applications, including inhibiting precancerous oral lesions (3). While generally safe at proper concentrations, prolonged or high-dose exposure can cause tissue irritation (3). However, repeated bacterial exposure to QACs has led to resistance. Genes such as *qacA*, *qacB*, and *gad* have been identified in staphylococci, and some pseudomonads tolerate concentrations of BAC used in disinfection (4).

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However, while primarily used in human medicine, BAC has occasional applications in veterinary settings.

Evaluating BAC's activity against pet oral bacteria, which are common in companion animals, is essential, as their microbiota differs from that of humans and may harbor resistant species (5). While BAC is occasionally applied as an oral antiseptic to these animals, its effectiveness and safety remain poorly studied (3). In a Spanish veterinary hospital, periodontal disease affected 60% of dogs and 31% of cats, followed by tooth resorption, gingivostomatitis, and oral tumors (6). Gingivitis, the reversible gum inflammation, may progress to periodontitis, which irreversibly damages supporting tooth structures (7). Plaque biofilms are highly resistant to antibiotics and antiseptics, so prevention via dental prophylaxis and home care—such as toothbrushing, dental diets, and oral rinses—is essential (7) (8). Feline oral health depends on a balanced microbiota of bacterial and fungal populations (9). Common bacterial genera in healthy cats include *Porphyromonas*, *Moraxella*, and *Fusobacterium*, with *Capnocytophaga canimorsus* predominating (9). Commensal bacteria coexist harmlessly, whereas pathogenic species can initiate disease (10). Disruption of this balance can lead to gingivitis, periodontitis, and systemic illness. Some oral bacteria also carry zoonotic potential, including *Bartonella*, *Pasteurella*, and *Salmonella*, which can infect humans through bites or close contact (11). To manage the oral bacteria, antiseptics like chlorhexidine, povidone-iodine, and QACs are commonly used (12) (13). Chlorhexidine is effective but can cause tissue irritation, staining, or taste aversion, and overuse may promote reduced susceptibility (12). QACs, including BAC, disrupt bacterial membranes effectively, yet veterinary-specific guidelines and safety data are limited (13).

Despite extensive research in humans, BAC's effectiveness against pet oral bacteria is understudied (12). Current veterinary antiseptics, while effective, have limitations, including side effects and resistance concerns. Investigating BAC as an alternative or supplemental antiseptic could improve oral health in pets and reduce zoonotic transmission. This study evaluates BAC's antimicrobial activity against bacteria from cats' oral cavities, providing insight into its potential role in veterinary dentistry (14).

METHODS AND MATERIALS

Materials

The materials used in this experiment included 80% benzalkonium chloride (Item No. 186241356987, eBay), at least five sterile general-purpose nutrient agar plates (ASIN B08C546HHK, Amazon), sterile cotton swabs (ASIN B0CQ24SV24, Amazon), inoculating loops (ASIN B01NAPK9XA, Amazon), sterile sensitivity disks (SKU LM-STERILE), and pipettes (ASIN B0C4JV8MZJ, Amazon). Clean containers were used to prepare the benzalkonium chloride dilutions, and an egg incubator capable of maintaining 37 °C (ASIN B0BZ4T53F1, Amazon) was used for bacterial incubation. Clean tap water was used for dilution. A 70% isopropyl alcohol solution or bleach solution was used to disinfect work surfaces and equipment. Disposable gloves (ASIN B0C9S5PMSD, Amazon) and a permanent marker (ASIN B08MBFPFCQ, Amazon) were also used. The detailed formulation of the general-purpose nutrient agar was not recorded. All materials were either sterilized before use or pre-sterilized, and procedures were conducted in a sanitized workspace to minimize contamination risk.

Safety and Sterility Procedures

All participants followed a strict contamination prevention protocol before handling materials. First, hands were thoroughly washed for at least 20 seconds to reduce the risk of introducing unwanted microorganisms to our procedure. Furthermore, workspaces were disinfected using either a 70% isopropyl alcohol or a bleach solution, both of which would eliminate contaminants in our workspace and limit unnecessary outside microbial interference. Particularly, the 70% alcohol solution was chosen because it falls in the optimal range for bacteria removal of 60 to 90%. Additionally, sodium hypochlorite, the active chemical in bleach, is extremely effective in destroying microbial cells – a major contributing factor to our selection of this disinfectant (2). After thoroughly sterilizing our experimental setting, disposable gloves were worn, and long hair was secured. All tools used in bacterial transfer, such as forceps and inoculating loops, were flame-sterilized, and new tools were used for each transfer to avoid cross-contamination. Petri dishes were clearly labeled with identifying information such as concentration levels and control groups, while plates were only partially opened when in use and were handled away from the face to prevent aerosol contamination. Upon completion of the experiment, plates were sealed in biohazard bags, sprayed with disinfectant, and discarded safely; all surfaces and equipment were disinfected again after the experiment. Bacterial density was not standardized using a McFarland turbidity standard, and lawn inoculation techniques were not strictly followed,

which may introduce variability in inhibition zone measurements.

Bacterial Collection and Cultivation

Bacterial samples were obtained by swabbing the teeth of a domestic cat using a sterile cotton swab. The swabbing was conducted using a circular brushing motion (x5) to ensure the collection of oral bacteria. The swab was then used to streak a sterile agar plate using standard streaking technique as demonstrated in video protocols (see Appendix or supplementary materials). Each agar plate was labeled appropriately and incubated upside down at 37 °C for 24 hours to allow bacterial colonies to grow. Bacterial density was not standardized using a McFarland turbidity standard, and lawn inoculation techniques were not strictly followed, which may introduce variability in inhibition zone measurements.

Identification and Isolation of General Bacteria and Antibacterial Testing

Following incubation, bacterial colonies suspected to be gram-positive cocci based on visual morphology were isolated. Bacterial identification was based solely on colony morphology, without confirmatory Gram staining, biochemical testing, or molecular analysis. Therefore, the specific bacterial species remain unidentified. Using a sterile inoculating loop, the isolated colonies were transferred onto two new sterile agar plates. Each plate was streaked in a consistent motion, and new samples from the original culture were used for each plate.

A series of benzalkonium chloride solutions was prepared at concentrations of 0.1%, 0.5%, 1%, 3%, and 5%. A 5% stock solution was first created by mixing 10 mL of 80% benzalkonium chloride with 190 mL of water. Subsequent dilutions were prepared as follows: 3%: 60 mL of 5% solution + 40 mL of water, 1%: 20 mL of 5% solution + 80 mL of water, 0.5%: 10 mL of 5% solution + 90 mL of water, 0.1%: 2 mL of 5% solution + 98 mL of water. Sterile sensitivity disks were then soaked in each of the five benzalkonium chloride concentrations. Using sterilized tweezers, the disks were placed on the two agar plates. The plate containing the 5%, 3%, and 1% concentrations received three disks arranged in a triangular formation to prevent overlap of diffusion zones. The second plate contained 0.5%, 0.1%, and a control disk (unsoaked). The reverse side of each plate was labeled to indicate disk placement and corresponding concentration.

All plates were incubated at 37 °C for 24 hours. Upon completion of the incubation period, each plate was photographed for documentation purposes. Photographs were taken under standardized lighting conditions on a dark, uncluttered background to reduce glare and improve clarity. These images were used to analyze and compare the antibacterial effects of each benzalkonium chloride concentration.

RESULTS

To determine the antibacterial effectiveness of benzalkonium chloride (BAC) against bacterial isolates, zones of inhibition were measured following 24 hours of incubation at 37 °C. The average diameters of the zones, taken from six trials per concentration, are reported in Table 1. Six technical replicates were conducted for each concentration across independently prepared agar plates. Zone diameters were measured manually using a ruler (mm scale).

Table 1. Average zone of inhibition for various BAC concentrations (values represent mean ± standard deviation).

BAC Concentration	Zone of Inhibition (mm)
5%	29.0 ± 0.8
3%	26.0 ± 1.1
1%	22.0 ± 0.7
0.5%	18.0 ± 0.5
0.1%	12.0 ± 0.9
Control (0%)	0.0 ± 0.0

There was a clear positive correlation between BAC concentration and inhibition zone size. The 5% solution exhibited the greatest antibacterial effect, with an average zone of 29 mm, followed closely by the 3% (26 mm) and 1% (22 mm) concentrations (Figure 1). At lower concentrations, 0.5% and 0.1% BAC produced smaller inhibition zones of 18 mm and 12 mm, respectively, while the control disk (0%) showed no inhibition (Figure 2).

These results indicate a descriptive trend suggesting a concentration-dependent increase, with higher BAC concentrations causing more pronounced bacterial suppression. Photographic evidence of representative plates supports the quantitative data.

Figure 1. *Antibacterial effects of benzalkonium chloride (BAC) at high concentrations (1%, 3%, and 5%) on bacterial isolates. Zones of inhibition increased with concentration, indicating greater bacterial suppression. Disks were arranged in a triangular formation on the agar plate and incubated at 37 °C for 24 hours.*

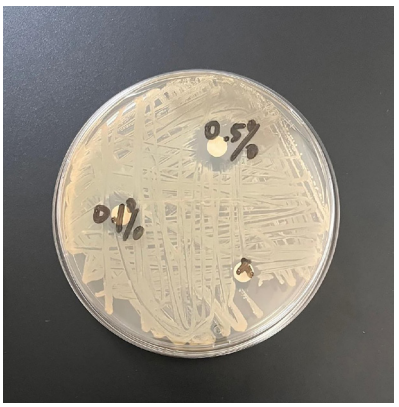

Figure 2. *Antibacterial effects of benzalkonium chloride (BAC) at lower concentrations (0.1% and 0.5%) compared to a control (0%) on bacterial isolates. Small inhibition zones are visible around the 0.1% and 0.5% disks, while the control shows no inhibition. The plate was incubated at 37 °C for 24 hours.*

DISCUSSION

The results of this study demonstrate a clear dose-dependent relationship between benzalkonium chloride (BAC) concentration and antibacterial activity. Average inhibition zones increased from 12 mm at 0.1% BAC to 29 mm at 5% BAC, showing a descriptive trend of increasing inhibition zone size with increasing concentration. This indicates that while antibacterial activity rises sharply at lower concentrations, the effect begins to plateau at higher concentrations, suggesting diminishing returns beyond 5%. Within the limited concentration range tested, 5% BAC appeared to be the most effective concentration, but further testing of higher concentrations would be necessary to determine whether bacterial suppression can be further optimized.

Several limitations should be considered. Oral sampling was performed on a privately owned domestic cat with owner consent. The procedure was non-invasive and involved external swabbing only. No institutional animal care protocol was required for this minimal-risk observational procedure; however, all efforts were made to ensure animal safety and minimize stress. Bacterial identification was based solely on morphology, without confirmatory Gram staining or molecular analysis. Additionally, bacterial density was not standardized, and formal statistical analyses were not conducted. The extended incubation period and unspecified agar formulation may also influence diffusion dynamics. Furthermore, minimum inhibitory concentration (MIC) values were not determined, limiting comparison to established antimicrobial benchmarks. Another major limitation of this study is that bacterial samples were obtained from a single domestic cat, which limits biological variability and prevents generalization to the broader feline oral microbiota.

Future studies should examine concentrations above 5% to assess whether the logarithmic relationship persists or reaches a plateau. Longer-term investigations could assess whether repeated exposure to BAC induces resistance, a key consideration given the known potential for resistance development with overuse of quaternary ammonium compounds. Incorporating molecular analysis of resistance genes (e.g., *qacA*, *qacB*) would offer more insight into the clinical utility and potential risks associated with BAC as an antiseptic.

Overall, this experiment provides preliminary evidence that BAC exhibits strong concentration-dependent antibacterial activity against oral bacteria, with a concentration of 5% emerging as the most effective within the tested range. However, further controlled studies with larger sample sizes, expanded concentration ranges, and resistance monitoring are necessary to confirm its potential role in veterinary or medical applications.

CONCLUSION

This study demonstrated that benzalkonium chloride (BAC) exhibits a strong concentration-dependent antibacterial effect against oral bacterial isolates, with

inhibition zones ranging from 12 mm at 0.1% to 29 mm at 5%. The results show a descriptive trend of increasing inhibition zone size with increasing concentration, 5% exhibited the highest observed antibacterial activity within the tested range, though higher concentrations may not yield proportionally greater inhibition. While these findings highlight BAC's potential as an oral antiseptic, limitations in methodology, sample size, and the in vitro nature of the study indicate that further investigation is needed. Future research should specifically assess the effects of concentrations higher than 5%, investigate the mechanisms and likelihood of bacterial resistance to BAC, and conduct in vivo studies to determine the safety and efficacy of BAC for clinical use in veterinary and medical settings.

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CONFLICT OF INTEREST

The authors do not have any conflict of interest related to the work.

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