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Original Article

Polymeric Antibiotic Beads for Canine Osteomyelitis: Advancing Localized Antibiotic Treatment with Enhanced Efficacy and Minimized Side Effects

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Abstract

Antibiotic beads represent a compelling treatment method for the treatment and prevention of canine osteomyelitis, as they provide localized antibiotic concentrations at the infection site with minimal systemic toxicity. The therapeutic efficacy of these beads has been demonstrated, highlighting their potential as a valuable local antibiotic therapy. In particular, the introduction of vancomycin - polymethylmethacrylate (PMMA) beads is highlighted as a promising new form of local antibiotic therapy. This article focuses on the use of antibiotic-impregnated beads to treat localized bacterial infections in the bones and joints of canines. Typically composed of poly(lactic-co-glycolic acid) (PLGA) or polymethylmethacrylate (PMMA), these beads contain antibiotics such as vancomycin, placed directly into the affected area, providing a high antibiotic concentration at the site of infection. These beads were developed for a slow antibiotic release over time; they ensure a constant protective effect against a broad spectrum of bacteria, demonstrating high efficacy and biocompatibility with the animal's body. This localized antibiotic treatment option offers several advantages over systemic antibiotics, such as reduced side effects and improved efficacy. It could be a promising option for the treatment of bone and joint infections in canines.

Keywords: ***Canine Osteomyelitis, Antibiotic-Impregnated Beads, Vancomycin, PMMA, PLGA***

Introduction

Osteomyelitis, an inflammation of bone and bone marrow due to bacterial infection, poses a significant challenge in veterinary medicine. In canines, this condition often arises when bacteria infiltrates the bloodstream and migrate to the bone and adjacent structures. The term "osteomyelitis" originates from the Greek words "osteon" (meaning bone) and "myelos" (meaning marrow), signifying an infection within the medullary portion of the bone.

To combat such bacterial infections, antibacterial beads have emerged as a promising solution, composed of materials that gradually release antibiotics over time. When strategically placed in wounds or surgical sites, these beads play a crucial role in inhibiting bacterial growth, consequently mitigating the risk of infection.

In veterinary practice, antibacterial beads find frequent application in scenarios where a heightened risk of infection exists, such as during surgeries or when treating open wounds. Furthermore, they prove instrumental in managing existing infections by direct placement into the affected areas.

These antibacterial beads, often containing antibiotics like vancomycin, DCM (Di-chloromethyl), PLGA (poly(lactic-co-glycolic acid)), and PMMA (Polymethyl methacrylate), are deployed to provide a concentrated drug delivery at the infection site. Conditions such as osteomyelitis, septic arthritis, and infected surgical wounds can be targeted either independently or in conjunction with other treatments like surgery, debridement (removal of infected tissue), and systemic antibiotics.

The design of these beads ensures a gradual release of antibacterial agents, maintaining a sustained protective level against bacteria. Key attributes, including high efficacy, bio-compatibility with the animal's body, and ease of administration directly into affected areas without extensive surgical procedures, underscore the practicality and utility of antibacterial beads in veterinary medicine.

These beads employ diverse antibacterial agents, such as silver ions, copper ions, triclosan, and quaternary ammonium compounds. Fabricated from polymers like PLGA 10-90, they can be impregnated with antibacterial agents, and in some cases, they are crafted from porous materials like zeolites or activated carbon to trap and eliminate bacteria. Additional components, including stabilizers, surfactants, or fillers, may be incorporated based on specific applications.

The composition of antibacterial beads varies according to their intended use and the antibacterial agent or material in use. Vancomycin, in particular, stands out as a preferred antibiotic due

to its broad-spectrum effectiveness against gram-positive bacteria, including methicillin-resistant Staphylococcus aureus (MRSA). Administered intravenously, the dosage is adjusted based on factors such as the animal patient's age, weight, and kidney function. This paper delves into the multifaceted aspects of antibacterial beads, exploring their design, applications, and the critical role of vancomycin in combating bacterial infections caused by open wounds in canines.

Materials and Method

In this study, the focus was on the systematic preparation of vancomycin-loaded beads, employing specific materials and a detailed method. The key materials used in the preparation of these antibiotic beads were vancomycin (Sigma-Aldrich, USA), Polymethyl methacrylate (PMMA) (Sigma-Aldrich,USA,) Dichloromethane (DCM) (Sigma-Aldrich, USA), Poly(lactic-co-glycolic acid) (PLGA) (Sigma-Aldrich, USA), distilled water, and polyester suture, USP 5/0 braided suture coated in wax. Constructed from polyester material with a smooth, unbroken finish. Attached to a 302 grade stainless steel needle with a 3/8 circle curvature. (Meril Life Sciences Pvt,. Ltd, India). The beads were designed with diameters of 3mm, 5mm, and 7mm, each consisting of 1mg of vancomycin and 5gm of PMMA respectively.

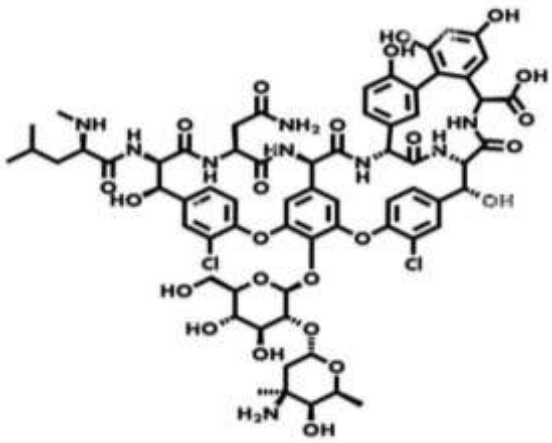
Vancomycin is a tricyclic glycopeptide antibiotic originally derived from the organism Streptococcus orientalis. Vancomycin is used to treat and prevent various bacterial infections caused by gram-positive bacteria, including methicillin-resistant Staphylococcus aureus (MRSA). It's primarily used to treat severe bacterial infections caused by Gram-positive bacteria, which are resistant to other antibiotics. Potency of vancomycine is high, toxicity is lower.

Gentamicin injection is used to treat serious bacterial infections in many different parts of the body. Gentamicin is an antibiotic that is commonly used to treat the following serious infections caused by Staphylococcus aureus, Enterobacter, Klebsiella, Serratia, Pseudomonas aeruginosa, bacteria. Blood infections (septicemia) Infections of the membranes surrounding the brain and spinal cord (meningitis). It is Broad-spectrum antibiotic effective against both Gram-positive and Gram-negative bacteria. Potency of gentamycine is moderate, toxicity is higher.

Vancomycin is primarily effective against Gram-positive bacteria, while gentamicin is effective against both Gram-positive and Gram-negative bacteria. Together, they can target a wider range of pathogens.the combination of these antibiotics can have a synergistic effect, meaning they work together effectively than when used individually. Both vancomycin and gentamicin have side effects, and using them together may increase the risk of toxicity.

2.1 Formulation of Vancomycin, PMMA, & PLGA Solution

In a first step, a homogeneous vancomycin solution was prepared by dissolving 1 mg vancomycin in distilled water. At the same time, a 10% PMMA solution was prepared from 10 g PMMA and 90 ml distilled water. In addition, the PLGA solution was prepared by dissolving 100 g of PLGA in DCM for 4 to 12 hours to ensure complete dissolution. The pre solution was stored for the subsequent steps. The molecular structure is illustrated in Figure 01.



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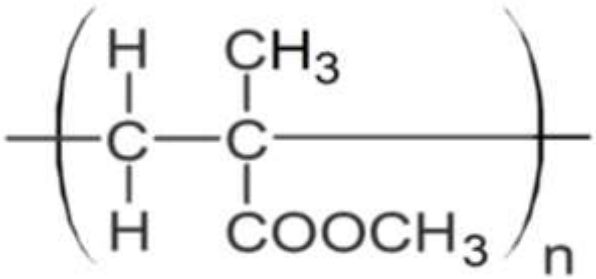

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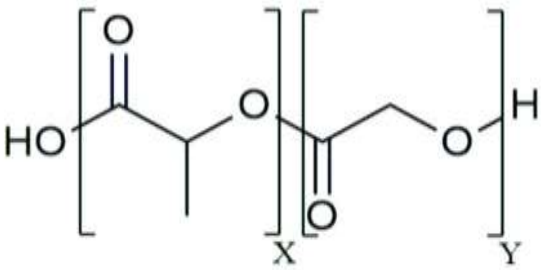


[c]

Figure 01.Vancomycin (C66H75Cl2N9O24) + (b) Polymethyl methacrylate (PMMA) (C5O2H8)n + (c) PLGA (Poly lactic-co-glycolic acid) (C5H8O5)n

2.2 Vancomycin-Loaded Beads Preparation through Solvent Evaporation and Moulding

Phase two involves combining the drug and polymer solutions. Vancomycin was gradually incorporated into the pre-mixed PLGA and PMMA solutions under constant agitation (e.g., for 60 minutes at 200-300 rpm) to ensure thorough blending and prevent air bubble formation. To fabricate the beads, a two-step procedure involving threading and solvent evaporation was employed. Polyester sutures, pre-cut to specific lengths, were threaded through the center of each bead, positioned within a silicone mould (refer to Figure 2 for visual representation). The combined solution was then poured into the mould, allowing the DCM solvent to evaporate gradually, resulting in the formation of Vancomycin-impregnated beads.

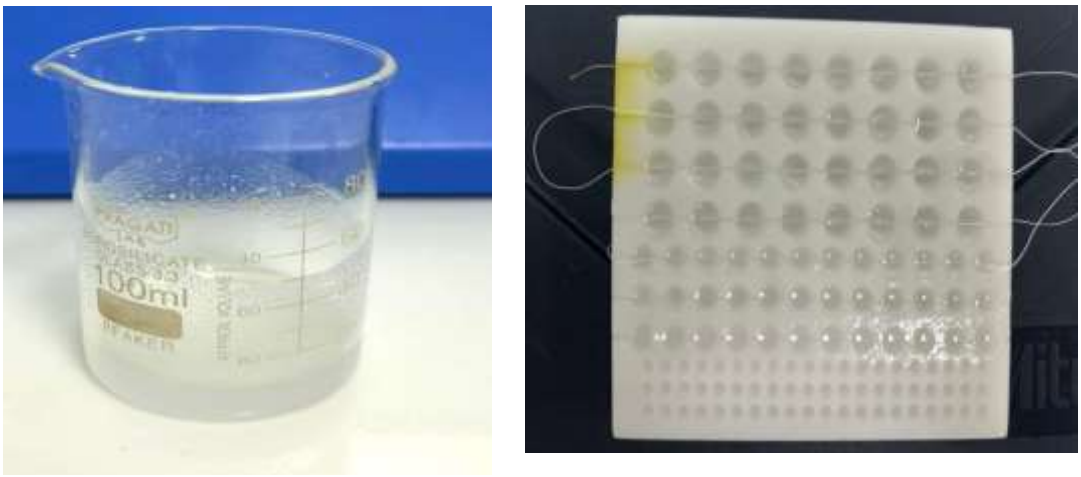


Figure 02.Evaporation and Moulding Process.

2.3 Beads Purification

To remove residual DCM from the beads, they were washed with distilled water, undergoing 3 repetitions of the washing process. DCM is water insoluble toxic solvent having boiling point just 40°C. We can remove this solvent with little heat and slight vacuum.

Detection of DCM in antibacterial beads:

The GC analysis uses a split injection mode with nitrogen carrier gas at 150°C and a 10:1 split ratio. The column is a 30-meter capillary column. The oven starts at 40°C for 4 minutes, then ramps to 200°C at 10°C/min and holds for 10 minutes. Total run time is 30 minutes. The GC column is a 30-meter fused silica capillary with a 0.32 mm inner diameter and 1.8 µm film thickness. It's a polar column (6% cyanopropyl/phenyl, 94% polydimethylsiloxane) equivalent to USP phase G43.

Preparation of standard solution:

Dichloromethane stock solution (1500 ppm):

Take accurately 75 mg of Dichloromethane in to the 50 ml standard volumetric flask and dilute up to the mark with N,N-Dimethylformamide. Concentration of the solution is 1500 ppm.

Dichloromethane standard solution (150 ppm):

Take 2 ml of Dichloromethane stock solution (1500 ppm) in 20 ml standard volumetric flask and dilute up to the mark with N,N-Dimethylformamide. Pipette 5 ml of this solution in 20 ml headspace vial and seal the vial immediately. Prepare six sample vial.

Preparation of sample solution

Transfer accurately 50 mg of sample in to the 20 ml headspace vial add 2 ml of N,N-Dimethylformamide and seal the vial immediately.

Subsequently, the beads were allowed to harden at room temperature for 45 minutes to achieve the desired firmness. With utmost care, they were then extracted from the mould. The aforementioned process yielded Vancomycin-embedded beads shown in the Figure 03, with the potential for medical applications

2.4 Endotoxin Detection Methodology for Antibiotic Beads: LAA Assay

In the assessment of antibiotic beads for Bacterial Endotoxin Testing (BET), the experimental methodology was employed. The beads were aseptically removed from mould. Subsequently, the beads were placed in a 500 ml depyrogenated beaker. An 80ml volume of LAL Reagent Water (LRW) was added to the beaker using a 100 ml depyrogenated measuring cylinder. The beaker was covered with aluminum foil, and the beads were incubated at 37°C ± 2°C for a minimum of 60 minutes in an orbital incubator shaker, serving as the test sample.As part of the production process, all glassware is depyrogenated at extremely high temperatures, well above the standard conditions of the depyrogenation process. These conditions ensure that the tubes are depyrogenated and suitable for performing the Bacterial Endotoxin Test (BET). As the molecular weight of the endotoxins is greater, they are removed or killed by depyrogenation, so we use depyrogenated glassware.

Following the incubation, the sample was diluted up to Maximum Valid Dilution (MVD)/4 in a depyrogenated test tube using LAL Reagent Water. Serial dilutions of Control Standard Endotoxin (CSE) in LRW were prepared in dilution tubes marked up to 4λ (λ = labeled LAL sensitivity). Each dilution was thoroughly mixed for at least 30 seconds before progressing to the next dilution.

For the assay, Negative Water Control (NWC), Positive Water Control (PWC), Negative Sample Control (NSC), and Positive Sample Control (PSC) assay tubes were prepared in duplicate. All assay tubes were then incubated in a heating block at 37.0°C ± 1.0°C for 60 ± 2 minutes. Post-incubation, the tubes were individually inverted by 180º C, and the positivity or negativity was determined based on gel formation.

The calculation of Maximum Valid Dilution (MVD) was performed as follows:

Calculation of the MVD

The endotoxin threshold is calculated as follows:

$$\text{Endotoxin limit} = \frac{K \times N}{V}$$

Where, K= The amount of endotoxin allowed per sample (20 EU/Beads)
N= The number of samples tested
V= The total volume of the extract or rinse

Result and Discussion

Antibacterial Beads Formation:

The solvent displacement technique successfully yielded a total number of 128 well defined vancomycin loaded beads. The beads possessed a spherical shape with a smooth surface and a white hue. Visual inspection and magnification examination confirmed a consistent diameter of approximately 3mm, 5mm, 7mm size across the bead population.

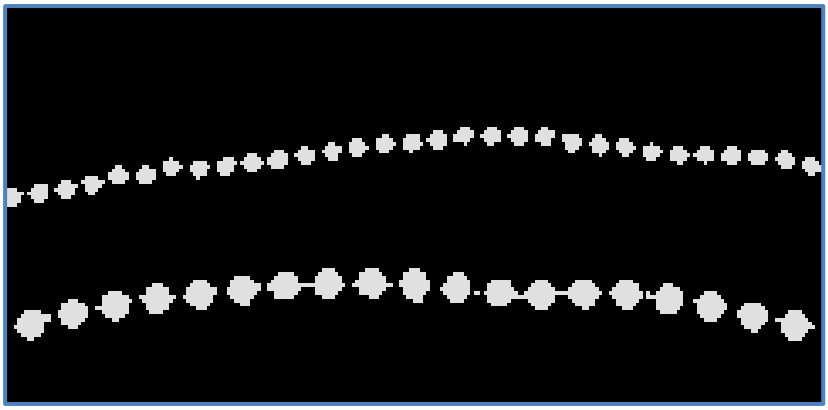


Figure 03: Close up view of Hardened Antibiotic Beads

Assessment of LAA Assay:

The LAA assay successfully determined the endotoxin levels in the antibiotic beads. The calculated endotoxin limit was 0.6 EU per bead, and the MVD was 1:20, corresponding to a maximum allowable detection limit of 0.03 EU per ml.

$$\text{Endotoxin limit} = \frac{20 \times 3}{100} = 0.6 \text{ EU}$$

The MVD is calculated as follows:

$$\text{Maximum Valid Dilution (MVD)} = \frac{\text{Endotoxin Limit}}{\text{Labelled Lysate Sensitivity}}$$

Endotoxin Limit = 0.6 EU/ ml
Lysate Label Claim sensitivity = 0.03 EU/ ml

$$\text{Maximum Valid Dilution (MVD)} = \frac{0.6}{0.03} = 20$$

$$\approx 1:20$$
$$\text{MVD/2} = 1:10$$
$$\text{MVD/4} = 1:5$$

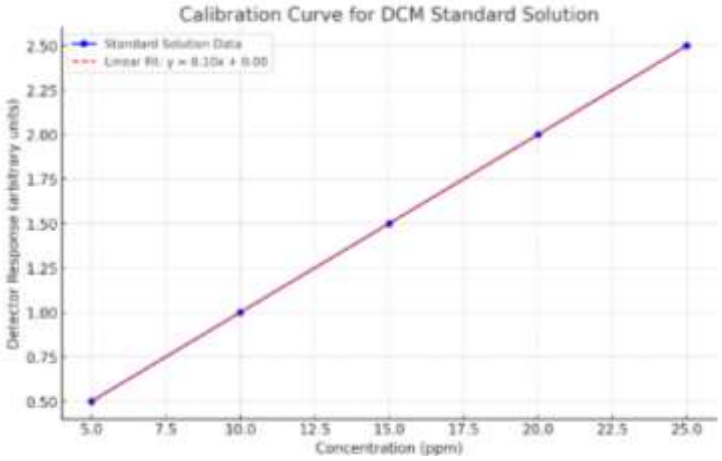
3.3 Dilution Table

Tube No.	Tube Content	LRW (µl)	CSE (µl)	Test Sample (µl)	LAL Reagent (µl)
1,2	NWC	100	–	–	100
3,4	PWC	50	50 (4λ)	–	100
5,6	NSC	50	–	50	100
7,8	PSC	–	50 (4λ)	50	100

NWC and NSC tubes showed no gel formation, confirming the absence of endotoxin contamination in the LAL reagent water and the test sample itself. Both PWC tubes displayed positive gelation, verifying the functionality of the lysate reagent and its sensitivity to endotoxin. In the PSC tubes, gel formation was observed in all replicates, indicating the presence of detectable endotoxin in the antibiotic beads. The LAA assay proved to be an effective method for detecting endotoxin in antibiotic beads. It offers several advantages over conventional BET methods, such as the rabbit pyrogen test, including increased sensitivity, specificity, and reduced animal experimentation. The observed gel formation in the PSC tubes suggests the presence of endotoxin in the beads, although further quantification is necessary to assess their suitability for clinical applications.Vancomycin and gentamicin are antibiotics used to treat serious bacterial infections. Vancomycin is effective against gram-positive bacteria, such as MRSA, and has a lower toxicity profile. After purifying the antibiotic beads, the dichloromethane (DCM) content in the beads was analysed using the gas chromatography (GC) method with 10 samples of antibiotic beads were prepared and analysed.

Quantitative Analysis of DCM Content:

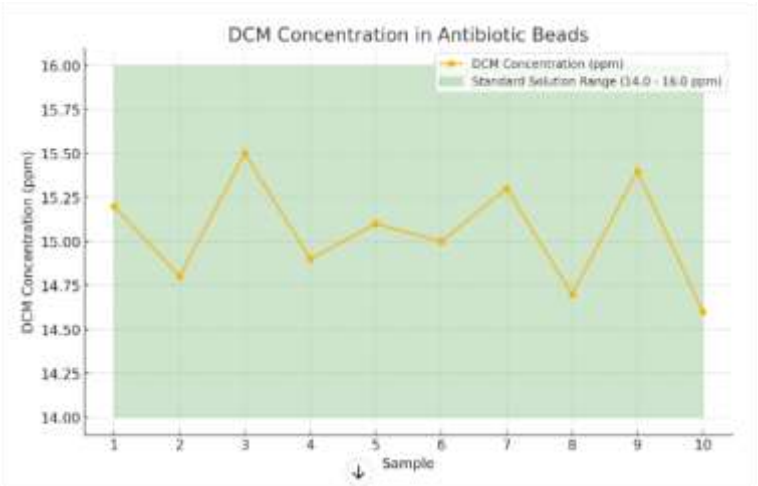
The consistent DCM concentrations within the acceptable range demonstrate the efficiency of the purification process in reducing residual solvent levels in the antibiotic beads. Maintaining DCM levels below the regulatory limit of 600 ppm ensures the safety of the antibiotics for clinical use.



Graph 1: The calibration curve (Figure 1) shows a linear relationship between DCM concentration and detector response, with a linear fit equation of y=0.1x+0.0. This linearity confirms the reliability of the GC method for quantifying DCM in the antibiotic beads. The blue line with markers represents the detector response for each known concentration of DCM. The red dashed line indicates the linear fit, showing the relationship between concentration and detector response.

The DCM content in the antibiotic beads was consistent across the samples, with an average concentration of approximately 15.0 ppm. All measured concentrations fell within the standard solution range of 14.0 - 16.0 ppm.

This quantitative analysis ensures that the purification process effectively reduces DCM levels to an acceptable range, ensuring the safety and efficacy of the antibiotic beads.



Graph 2: Illustrates the DCM concentration in the antibiotic beads for each sample, along with the standard solution range (14.0 - 16.0 ppm). The green shaded area represents the acceptable standard range, and the blue line with markers represents the DCM concentration for each sample.

These results underscore the importance of rigorous quality control measures in the production of antibiotic beads. The established calibration curve provides a robust method for ongoing monitoring of residual solvents, contributing to the overall quality assurance of antibiotic formulations.

Conclusion

In conclusion, antibiotic beads represent a compelling and effective therapeutic strategy for the treatment and prevention of osteomyelitis in canines, as they deliver localized antibiotic concentrations with minimal systemic toxicity. Our study shows that the above mentioned antibiotics vancomycin and gentamicin as individual or as mixture show quite decent therapeutic efficacy. Demonstrated therapeutic efficacy underscores their potential as a valuable local antibiotic therapy, with particular emphasis on the innovation of vancomycin polymethyl methacrylate (PMMA) bead delivery. This research is specifically investigating the use of antibiotic-impregnated beads, consisting mainly of poly (lactic-co-glycolic acid) (PLGA) or PMMA, for the targeted treatment of local bacterial infections in the bones and joints of canines. These antibiotic vancomycin enriched beads could be strategically placed at the affected site and provide a sustained high concentration of antibiotic at the infection site. These beads were designed for gradual release; they provide consistent protection against a broad spectrum of bacteria, showcasing remarkable efficacy and biocompatibility. The localized nature of this antibiotic treatment offers advantages over traditional systemic approaches, including reduced side effects and improved efficacy, positioning it as a promising option for the treatment of bone and joint infections in canines. Furthermore, the focus on antibiotic beads opens avenues for future research and development. The methodology employed in investigating their use for Bacterial Endotoxin Testing (BET) as a sterility test, with a demonstrated Maximum Valid Dilution (MVD) of 1:20, attests to the reliability of the testing process. As we advance, this approach inclusive of Negative Water Control (NWC), Positive Water Control (PWC), Negative Sample Control (NSC), and Positive Sample Control (PSC) assay tubes, adheres to stringent criteria for interpretation. The set endotoxin limit of not exceeding 20 EU per beads ensures the continued safety and effectiveness of antibiotic beads in the localized treatment of canine osteomyelitis, paving the way for future advancements in this promising therapeutic avenue.

References

[1] Wahlig, H., Dingeldein, E., Bergmann, R., & Reuss, K. (1978). The release of gentamicin from

[2] polymethylmethacrylate beads. An experimental and pharmacokinetic study. *The Journal of bone and joint surgery. British volume*, *60-B*(2), 270–275. https://doi.org/10.1302/0301-620X.60B2.659478

[3] Cunningham, A., Demarest, G., Rosen, P., & DeCoster, T. A. (2000). Antibiotic bead production. *The Iowa orthopaedic journal*, *20*, 31–35.

[4] Gomes, D., Pereira, M., & Bettencourt, A. F. (2013). Osteomyelitis: an overview of antimicrobial therapy. *Brazilian Journal of Pharmaceutical Sciences*, *49*, 13-27.

[5] Olsen, R. S., Sawyere, D. M., Davis, J. L., Lanz, O. I., & Werre, S. R. (2023). Bead size has a greater effect on in vitro elution from antimicrobial-impregnated calcium sulfate beads than drug concentration. *American journal of veterinary research*, *84*(5), ajvr.22.12.0216. https://doi.org/10.2460/ajvr.22.12.0216

[6] Lindsay C. Peterson DVM, Stanley E. Kim BVSc, MS, DACVS, Daniel D. Lewis DVM, DACVS, Matthew D. Johnson DVM, MS, DACVS, Cassio R. A. Ferrigno DVM, PhD, MS

[7] First published: 25 January 2021 https://doi.org/10.1111/vsu.13570

[8] 6) Peterson, L. C., Kim, S. E., Lewis, D. D., Johnson, M. D., & Ferrigno, C. R. A. (2021). Calcium sulfate antibiotic-impregnated bead implantation for deep surgical site infection associated with orthopedic surgery in small animals. *Veterinary surgery : VS*, *50*(4), 748–757. https://doi.org/10.1111/vsu.13570

[9] FSeligson, D., Popham, J., Voos, K., Henry, S.L.,Paguri, M.: Antibiotic leaching from polymethylmethacrylate beads. *J Bone Joint Surg*, 75A(5): 714-720, 1993.

[10] Henry, S.L., Ostermann, P.A.W., Seligson, D.: The prophylactic use of antibiotic impregnated beads in open fractures. *J Trauma* 30: 1231-1238, 1990.

[11] Goodell, J.A., Flick, A.B., Hebert, J.C., and Howe, J.G.: Preparation and release characteristics of tobramycin impregnated polymethylmethacrylate beads. *Am J Hosp Pharm*, 43: 1454-1460, 1986.

[12] LAMP, K.C.; FRIEDRICH, L.V.; MENDEZ-VIGO, L.; RUSSO, R. Clinical experience with daptomycin for the treatment of patients with osteomyelitis. *Am. J. Med*., v.120, n.10, suppl.1, p.S13-S20, 2007.

[13] MARRA, F.; ROBBINS, G.M.; MASRI, B.A.; DUNCAN, C.; WASAN, K.M.; KWONG, E.H.; JEWESSON, P.J. Amphotericin B-loaded bone cement to treat osteomyelitis caused by Candida albicans. *Can. J. Surg.*, v.44, n.5, p.383-386, 2001.

[14] KANELLAKOPOULOU, K.; GIAMARELLOS-BOURBOULIS, E.J. Carrier systems for the local delivery of antibiotics in bone infections. *Drugs*, v.59, n.6, p.1223-1232, 2000.

[15] AMBROSE, C.G.; CLYBURN, T.A.; LOUDEN, K.; JOSEPH, J.; WRIGHT, J.; GULATI, P.; GOGOLA, G.R.; MIKOS, A.G. Effective Treatment of osteomyelitis with biodegradable microspheres in a rabbit model. *Clin. Orthop. Relat. Res*., v.421, p.293-299, 2004.

[16] Santschi EM, McGarvey L. In vitro elution of gentamicin from plaster of Paris beads. *Vet Surg.* 2003;32(2): 128–133. doi:10.1053/jvet.2003.50010

[17] SHIRTLIFF, M.E.; CALHOUN, J.H.; MADER, J.T. Experimental osteomyelitis treatment with antibiotic-impregnated hydroxyapatite. *Clin. Orthop. Relat. Res.*, v.401, p.239-247, 2002.

[18] SAMUEL, S.; MATHEW, B.S.; VEERARAGHAVAN, B.; FLEMING, D.H.; SAMUEL, B.; CHITTARANJAN, S.B.; PRAKASH, J.A. *In vitro* study of elution kinetics and bio-activity of meropenem-loaded acrylic bone cement. *J. Orthopaedics Traumatol*., v.13, n.3, p.131-136, 2012.