In vivo test of a nanostructured formulation for control of bovine mastitis - <u>Guimarães</u> <u>A.S.</u><sup>1\*</sup>, Gern J.C.<sup>1</sup>, Lange C.C.<sup>1</sup>, Andrade P.V.D.<sup>1</sup>, Mendonça L.C.<sup>1</sup>, Araujo R.S.<sup>2</sup>, Mosqueira V.C.F.<sup>2</sup>, Brito M.A.V.P.<sup>1</sup>, Brandão H.M.<sup>1\*</sup>

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Mastitis is the disease that causes the most economic losses in dairy herds worldwide. Its treatment consists of parenteral or intramammary antibiotic therapy, the last route is the most efficient. However, cases of treatment failure are common. To reduce these limitations, this study aimed to evaluate the potential use of a nanostructured formulation for the treatment of mastitis in dairy cattle. Nanocapsules containing cloxacillin were prepared by interfacial deposition of preformed polymer, followed by evaporation of the solvent. Three cows (twelve mammary quarters) at drying off were treated with 600 mg of nanostructured cloxacillin for intramammary route. No pathogen was recovered from the treated quarters, even after three microbiological tests. In turn, the mammary glands that were free of pathogenic bacteria remained healthy during the experimental period. The formulation presented both curative and preventive effect in mammary quarter, indicating its promising use to control this infection.

Key-words: cloxacillin, intramammary therapy, nanocapsules

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### INTRODUCTION

The Bovine mastitis causes economic losses in all segments of the milk production chain, reducing productivity by increasing the cost of production, the number of somatic cells and reducing the quality of milk [1]. Within a production system, such consequences result in lower profits, mainly due to the current payment system adopted by most companies that buy milk [2]. In addition, the physico-chemical changes in milk from animals with mastitis reduce industrial output, cause industrial processing problems and lead to poor quality products [2].A wide variety of microorganisms may cause mastitis, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Staphylococcus aureus and Escherichia coli are considered the most important [3]. For the treatment and prevention of new infections with these pathogens is recommended the dry cow therapy, which consists of intramammary infusion of long-acting formulations containing antibiotic at the time of drying (ie, 45-60 days before calving). However, recurrences are frequent after therapy, especially when pathogens can survive within abscesses, epithelial cells, phagocytic cells and alveolar ducts and obstructed by blood clot, places where the drug does not arrive [1]. In order to overcome this limitation, our team has developed a formulation containing intramammary nanocapsules able to direct the antibiotic to the surface of the glandular epithelium and the inside of phagocytic cells.

In this context, the objective of this study was to evaluate the clinical use of nanocapsules containing intramammary cloxacillin for the treatment of cows naturally infected with pathogens that cause mastitis.

### MATERIALS AND METHODS

solvent, according to the methodology previously described for [4]. The average size and polydispersity index (PDI) of the particles were determined by photon correlation spectroscopy at 20°C in a Nanosizer N5Plus Analyser Beckmann Coulter (Fullerton, USA), while the zeta potential was determined by laser Doppler anemometry in a Zetasizer HS3000 (Malvern Instruments, Malvern, UK).

Determination of the health status of the mammary quarter was held by three bacterial isolates [5,6]. Mammary quarter was infected when the pathogen was isolated at least twice

#### RESULTS AND DISCUSSION

The nanocapsules containing cloxacillin infused in the mammary glands had on average diameter of 322nm and a low polidispersal with 0.088 PDI. The zeta potential was estimated at -28mV, indicating that the particles can be stable in aqueous suspension by electrostatic repulsion, once the magnitude of zeta potential is very close to 30mV [7].

In the infected mammary quarters were isolated Corynebacterium spp. (03), coagulase negative Staphylococcus (02) and Streptococcus uberis (01) that, when treated, become free of pathogens (Table 1).

Table 1. Microbiological status of mammary quarters treated with 600 mg of nanocoated cloxacillin.

Mammary quarters	Pre treatment		Post calving	
	Neg	pos	neg	pos
With mastitis	-	06	06	-
Without mastitis	06	-	06	

In turn, the mammary glands that were presented free of pathogenic Nanocapsules containing cloxacillin were prepared by interfacial bacteria remained healthy during the experimental period. These deposition of preformed polymer, followed by evaporation of the results indicate that the nanoparticulate system used in the experiment presented both preventive and curative effect for bovine mastitis

> The period near calving, marked by the drying stage and the first 30 days of lactation, is responsible for the higher incidence of new infections in the mammary gland. This is due to the greater vulnerability of the gland, which can fail in maintaining the keratinous plug in the ostium of the teat and reduced immune responsiveness of the mammary gland [8]. Consequently, a good anti-mastitis formulation should be effective curative and preventative when applied at the time of drying [9].

## CONCLUSION

The results, although preliminary, indicate the promising use of nanocoated cloxacillin in dry cow therapy. However, to confirm these findings and provide security for its use, the number of treated animals should be extended.

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