**Biodistribution of nanocapsules in bovine mammary gland model** - Lange C.C.[1], Souza G.N.[1], Castanheira R.G.[2], Brandi R.R.[1,3], Mosqueira V.C.F.[1], Silva S.R.[1,3], Gern J.C.[1], Andrade P.V.D.[4], Mendonça L.C., Calvinho L.F.[5], Guimarães A.S.[1], Brandão H.M.[1*]

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The determination of the biodistribution of pharmaceutical formulations is important to ensure that the drug reaches all the tissue to be treated. Recent studies demonstrated that udder *ex situ* may be used to assess the biodistribution of intramammary formulations. We used this model to evaluate the biodistribution of nanocapsules containing cloxacillin, a formulation capable of directing the antibiotic into the polymorphonuclear (PMN) and, thus, improve cure rates in cases of mastitis caused by pathogens resistant to phagocytosis. Nanocapsules containing cloxacillin were prepared by interfacial deposition of preformed polymer, followed by evaporation of the solvent. However, it was used chitosan conjugated to fluorescein isothiocyanate (FITC) to replace conventional chitosan. Four mammary glands of crossbred cows were collected at the slaughter. After removal of the gland, the external pudendal artery was cannulated of each udder quarter, which were perfused with 1 L of sodium citrate 0.9% (w/v), cooled and brought to the laboratory. It was possible to observe the presence of nanocapsules on the back of the glands, 15 cm from the base of the teat, which demonstrates that the nanocapsules have potential to spread to the entire gland. In a short time the nanocapsules reached ducts, demonstrating that there was no accumulation in the gland cistern. These results indicate good tissue biodistribution, one of the prerequisites for intramammary formulations.

Key-words: intramammary formulation, cloxacillin, mastitis therapy

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# Biodistribution of nanocapsules in bovine mammary gland *ex situ*

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## INTRODUCTION

The biodistribution of drugs in an *ex situ* experimental model have been used as an alternative to the use of animals. This is due to growing concern about the ethics and animal welfare [1]. Kietzmann and Ehinger [2] demonstrated the feasibility of using *ex situ* mammary glands for studies of diffusion kinetics of antibiotics in cattle. For this purpose, the authors evaluated the tissue biodistribution of benzathine penicillin after intramammary administration. In other studies, the same group [3,4,5] used the same model to assess the tissue biodistribution of other antibiotics, for example cloxacillin, cefquinome, oxacillin and ampicillin.Our team has developed a formulation comprising nanocapsules, capable of directing the antibiotic into the interior of polymorphonuclear (PMN) and, thus, improve cure rates in cases of mastitis caused by pathogens resistant to phagocytosis. However, when nanoencapsulated, the antibiotic may have their biodistribution modified, with the possibility of nanocapsules do not reach the tops of the mammary gland. Therefore, this project aims to evaluate the biodistribution of nanocapsules in models of the bovine mammary gland *ex situ*.

## MATERIALS AND METHODS

Nanocapsules containing cloxacillin were prepared by interfacial deposition of preformed polymer, followed by evaporation of the solvent, observing the details of the methodology previously described by Mosqueira et al [6]. However, it was used chitosan conjugated to fluorescein isothiocyanate (FITC) to replace conventional chitosan, for this, 100mg of FITC was added to 150 mL of methanol and subsequently mixed to 100 mL of 1% chitosan in 0,1 M $CH_3COOH$. After three hours of reaction in the dark environment, chitosan labeled with FITC was precipitated by raising the pH. For removal of free FITC, the precipitate was subjected to repeated cycles of washing and centrifugation (40.000 xg for 10 min) until no fluorescence was detected in the supernatant (NanoDrop Spectrometer Thermo Scientific).

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).

Four mammary glands of crossbreed cows were collected at the slaughter. After removal of the gland, the external pudendal artery was cannulated of each udder quarter, which were perfused with 1 L of sodium citrate 0.9% (w / v) cooled and brought to the laboratory. Following procedures previously described by Kietzmann e Ehinger [2], glands were maintained mimicking the animal position for six hours tissue viability was achieved through the infusion of 120ml/min Tyrodi solution, previously heated to 41°C and oxygenated with carbogen. In each mammary quarter was injected 600mg of cloxacillin nanocoated and, at the end of six hours, tissue samples were collected 5, 10 and 15cm from the base of the teat for the preparation of histological sections on a freezing microtome (HYRAX-25, Carl Zeiss) and subsequent evaluation by fluorescence microscopy (Axioplan I Carl Zeiss).

## RESULTS AND DISCUSSION

In FIG. 1A is possible to observe a slight autofluorescence in a histological section of the mammary gland 10 cm from the base of the teat without treatment with nanocapsules.

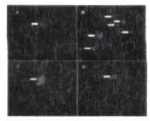

**Figure 1. A.** Fluorescence microscopy (10 cm far from the base of the teat) control group. **B.** Fluorescence microscopy of mammary gland treated with 600 mg of cloxacillin nanocoated, 5 cm far from the base of the teat. Fluorescence microscopy of mammary gland treated with 600 mg of cloxacillin nanocoated, 10 cm far from the base of the teat. **D.** Fluorescence microscopy of mammary gland treated with 600 mg of cloxacillin nanocoated, 15 cm far from the base of the teat. Arrows indicate ducts. FIG. 1B represents a histological section distant five centimeters from the base of the teat of a mammary quarter that received the treatment. In this, we can see lots of ducts with intense fluorescence (arrows). Deeper in the glandular tissue (Fig. 1C and 1D), 10 and 15cm away from the base of the teat, respectively, with intense fluorescence were observed.

The bovine mammary gland is composed of a large amount of glandular acini, which produce milk.This is drained into the teat cistern through a vast network of ducts that ramify throughout the parenchyma to the glandular acini and deposit in the teat cistern. When administered via intramammary, an antibiotic does exactly the opposite way of the milk by filling the tank first, then the internal space of the upper and lower ducts, and then reach the glandular acini. In just six hours after administration, the nanocapsules have reached the most distal area of the mammary gland, indicating its fast and good tissue biodistribution.

## CONCLUSION

In a short time the nanocapsules peaked galactophorous ducts, demonstrating that there was no accumulation of naocapsules in the gland cistern. These results indicate good tissue biodistribution, one of the prerequisites for intramammary formulations.

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