Cloxacillin nanostructured formulation for control of clinical bovine keratoconjunctivitis - Guimarães A.S.[1*], Lange C.C.[1], O`Connor A.M.[2], Castanheira R.G.[3], Mosqueira V.C.F.[3], Souza G.N.[1], V.C.F.[3], Mendonça L.C.[1], Brito M.A.V.P.[1], Gern J.C.[1], Brandão H.M.[1]

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Infectious bovine keratoconjunctivitis or `pinkeye' is a widespread, contagious ocular disease of cattle, mainly dairy cattle, that is caused by the Gram negative bacterium *Moraxella bovis.* Its treatment consists of parenteral or topic antibiotic therapy, the last route is used in lactating cows to reduce milk discart. However, cases of treatment failure are common, because the lacrimation removes the antibiotic. To reduce these limitations, this study aimed to evaluate the potential use mucoadhesive cloxacillin nanoparticles for the treatment of keratoconjunctivitis in dairy cattle. To test these nanoparticles, two cows naturally infected were treated with 1.0 mL (with 0.32 mg of nanostructured cloxacillin) for ocular route. *Moraxella bovis* was isolated by molecular method before the beginning of treatment that began when there was intense tearing and presence of visible corneal ulcer. The animals were treated every 12 hour for six days. The cure was considered by absence of clinicals symptoms and the bacterium after treatment. The mucoadhesive nanoparticle-based formulation promoted clinical cure using low doses of antibiotics, probably due to the sustained release of the antibiotic which kept the minimum inhibitory concentration in the eyes. The results indicate the promising use of nanocoated cloxacillin to control infectious bovine keratoconjunctivitis.

Key-words: *Moraxella bovis*, infectious keratoconjunctivitis, mucoadhesive nanoparticles

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Cloxacillin nanostructured formulation for control of clinical bovine keratoconjunctivitis

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INTRODUCTION

Infectious bovine keratoconjunctivitis (IBK; pinkeye) is the most common ocular disease of cattle; affected animals exhibit corneal ulceration leading to corneal scarring of [1]. Bovine keratoconjunctivitis is a known disease of cattle breeders in different regions of the world. It affects the eyes of dairy or beef cattle, regardless of age. However calves and confined animals are among the main victims of the disease. The infection rarely results in death, but losses with infected animals can be significant [1]. *Moraxella bovis* is the main etiological agent of bovine keratoconjunctivitis. The first reports linking a bacterium with infectious bovine keratoconjunctivitis were performed by [3]. In Brazil, [4] was a pioneer in linking *M. bovis* to bovine keratoconjunctivitis. For the treatment of new infection with these pathogens use of topical treatment with antibiotics is recommended. The treatment consists of the aplication of formulations containing antibiotic during the infection (clinical signs). However, recurrences can occur, especially due to treatments carried out incorrectly or with ineffective antibiotics [5, 6]. In order to overcome this limitation, our team has developed a formulation containing nanocapsules able to direct the antibiotic to the ocular surface with greater ability to adhere. In this context, the objective was to evaluate the clinical use of nanocapsules containing cloxacillin for the treatment of cows with clinical infectious keratoconjunctivitis.

MATERIALS AND METHODS

Nanocapsules containing cloxacillin were prepared by interfacial deposition of preformed polymer, followed by evaporation of the solvent, according to the methodology previously described for [7].
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).
An Outbreak of clinical ceraconjuntivite in cattle occurred in the beginning of June 2012 in a Holstein herd in Juiz de Fora, Minas Gerais.
The treatment began when there was intense lacrimation and the presence of an ulcer with 1 cm^2. Two animals were treated for six days at intervals of 12 h. After treatmene, the clinical evaluation was performed based on the healing of ulcers and absence of tearing. Before treatment, swab was used to collect material from all animals to identify the genus and species present in the outbreak.

RESULTS AND DISCUSSION

The nanoparticles containing cloxacillin used in topic the treatment had an 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 [8].
Moraxella bovis was isolated by molecular method [9,10] before the beginning of treatment an the treated animals presented visible clinical and microbiological cure in 6 days of treatment.

Cloxacillin nanoestructured formulation showed curative and preventative effect when applied locally in the eyes of the affected animals. Nanoparticle-based products require lower effective dose and have better adherence to ocular mucosa, reducing losses in the minimum inhibitory concentration by tearing, very common in these cases.

CONCLUSION

The results, although preliminary, indicate the promising use of cloxacillin nanocoated to infectious bovine keratoconjunctivitis. However, to confirm these findings and provide security for its use, the number of treated animals should be increased.

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