Evaluation of a nanopropolis for control of clinical bovine keratoconjunctivitis - Guimarães A.S.[1]*, Lange C.C.[1], O`Connor A.M.[2], Raposo N.R.B.[3], Silva S.R.[4], Brandi R.R.[4], Brito M.A.V.P.[1], Souza G.N.[1], Gern J.C.[1], Brandão H.M.[1]

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Organic milk is produced without synthetic antibiotics that may be a limitant to treat a lot of infections. An alternative in organic systems to treat infections is propolis, a natural antibacterial and anti-inflammatory product. Recently, we demonstrated that propolis in nanoscale have the antibacterial effect enhanced against *S. aureus*. Here, we use nanopropolis to treat cows naturally infected with *Moraxella bovis*. This bacterium causes keratoconjunctivitis and affected animals have impaired vision and lowered weight gains, which reduces their economic value. To test these nanoparticles, four heifers were treated with 1.0 mL (3.6 mg/mL) of nanopropolis for ocular route, both eyes. The treatment started when there was heavy presence of tearing and visible corneal ulcer. The animals were treated at 8 hour intervals for six days. The treated animals showed clinical (healing of the ulcers and absence of tearing) and microbiological cure among five and six days of treatment. *Moraxella bovis* was isolated by molecular method before the beginning of treatment and its absence was confirmed after that. The results indicate the promising use of nanopropolis to control infectious bovine keratoconjunctivitis and this new nanoparticle can be a alternative of natural antibiotic to use by the organic milk producer.

Key-words: *Moraxella bovis*, nanopropolis, infectious keratoconjunctivitis

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Evaluation of a nonoparticle formulation of propolis for control of clinical bovine keratoconjunctivitis

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INTRODUCTION

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. Infectious bovine keratoconjunctivitis (IBK; pinkeye) is the most common ocular disease of cattle; affected animals exhibit corneal ulceration leading to corneal scarring of [1]. Calves and confined animals are among the main victims of the disease. The infection rarely results in death, but their losses can be significant [2]. The *Moraxella bovis* is the etiological agent of bovine keratoconjunctivitis. The first reports of Gram-negative with infectious bovine keratoconjunctivitis were performed by [3], [4] [5] and [6]. In Brazil, [7] was a pioneer in linking the *M. bovis* to bovine keratoconjunctivitis. For the treatment of new infection with these pathogens is recommended use of topical treatment with antibiotics, which consists of aplication formulations containing antibiotic at the time of infection. However, recurrences can occur, especially due to treatments carried out incorrectly or with antibiotics ineffective [8]. Our team has developed a formulation containing nanocapsules able to direct the antibiotic to the ocular surface and greater capacity for adherence. In this context, the objective of this study was to evaluate the clinical application of nanocapsules containing propolis for the treatment of cows with clinical infectious keratoconjunctivitis.

MATERIALS AND METHODS

Outbreak of clinical ceraconjuntivite in cattle occurred in the beginning of June 2012 in Holstein herd located in Juiz de Fora, Minas Gerais.

Treatment began when there was intense lacrimation and presence of ulcer with 1 cm^2. Four animals were treated for six days at intervals of 12 h, for such they were properly restrained, where 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 species present in the outbreak.

Nanocapsules containing propolis were prepared by interfacial deposition of preformed polymer, followed by evaporation of the solvent, according to the methodology previously described for [8, 9].

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).

RESULTS AND DISCUSSION

The nanoparticles containing propolis used in topic treatment had 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 [10].

Moraxella bovis was isolated by molecular method [11,12] before the beginning of treatment an the treated animals showed clinical and microbiological cure in six days of treatment.

Propolis nanoparticles formulation should be effective, for treatment and prevention, when applied locally in the eyes of infected 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 effectiveness of propolis nanocoated to infectious bovine keratoconjunctivitis. However, to confirm these results and provide security for its use, the number of treated animals should be incresead.

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