

Molecular cloning and expression of epsilon toxin from *Clostridium perfringens* type D and tests of animal immunization

A.M. Souza^{1,2}, J.K.P. Reis³, R.A. Assis⁴, C.C. Horta¹, F.F. Siqueira¹, S. Facchin¹, E.R. Alvarenga¹, C.S. Castro¹, F.M. Salvarani², R.O.S. Silva², P.S. Pires², C. Contigli⁵, F.C.F. Lobato^{2*} and E. Kalapothakis^{1*}

¹Laboratório de Marcadores Moleculares e Biotecnologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil

²Laboratório de Anaeróbios, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil

³Laboratório de Retrovírus da Escola de Veterinária, Belo Horizonte, MG, Brasil

⁴Laboratório Nacional Agropecuário/Pedro Leopoldo/Minas Gerais, Ministério da Agricultura, Belo Horizonte, MG, Brasil

⁵Fundação Centro Tecnológico de Minas Gerais (CETEC), Belo Horizonte, MG, Brasil

*These authors contributed equally to this study.

Corresponding author: E. Kalapothakis

E-mail: ekalapo@icb.ufmg.br

Genet. Mol. Res. 9 (1): 266-276 (2010)

Received October 13, 2009

Accepted November 9, 2009

Published February 18, 2010

ABSTRACT. Epsilon toxin produced by *Clostridium perfringens* types B and D causes enterotoxemia in sheep, goats and calves. Enterotoxemia can cause acute or superacute disease, with sudden death of the affected animal. It provokes huge economic losses when large numbers of livestock are affected. Therapeutic intervention is challenging, because the disease progresses very rapidly. However, it can be prevented by immunization with specific immunogenic vaccines. We cloned the *etx* gene, encoding epsilon toxin, into vector pET-11a; recombinant epsilon toxin (rec- ϵ) was expressed in inclusion bodies and was used for animal immunization. Serum protection was evaluated and cross-serum neutralization tests were used to characterize the recombinant toxin. To analyze the potency of the

toxin (as an antigen), rabbits were immunized with 50, 100 or 200 µg recombinant toxin, using aluminum hydroxide gel as an adjuvant. Titers of 10, 30 and 40 IU/mL were obtained, respectively. These titers were higher than the minimum level required by the European Pharmacopoeia (5 IU/mL) and by the USA Code of Federal Regulation (2 IU/mL). This rec-ε is a good candidate for vaccine production against enterotoxemia caused by epsilon toxin of *C. perfringens* type D.

Key words: *Clostridium perfringens*; Epsilon toxin; *etx* gene; Vaccine; Enterotoxemia