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Service (sector) Cornea and External Disease Nº CEP

VALIDATION OF THE PIG MODEL OF ACANTHAMOEBA KERATITIS

Autors: Yeh SI, Alvarenga LS, Branco BC, Yu MCZ, Martins MC, Hofling-Lima AL, Foronda AS, Freitas D Purpose: To reproduce the pig model of Acanthamoeba keratitis Methods: Six female pigs weighing approximately 20 kg were used in the study. Animals were handled in accordance with the Association for Research in Vision and Ophthalmology (ARVO) resolution on the Use of Animals in Research. All corneas were examined prior to each experiment to exclude any host with preexisting lesions. Acanthamoeba castellani , originally isolated from a patient with amoebic keratitis, was obtained from American Type Culture Collection (ATCC). Parasites were grown in axenic cultures containing peptone-yeast-glucose medium in test tubes at 25oC. Sterile hydrophilic soft contact lenses (SCL; PureVision® – Bausch & Lomb) were incubated (25oC for 24 hr) with A. castellani in 1.0 ml PYG medium at a concentration of 3 x 105 organisms / ml (90% trophozoites, 10% cysts) in sterile tissue culture cluster wells. Pigs were anesthetized with 5% mixture of isoflurane and 95% oxygen delivered by an inhalator. The corneal surface was gently abraded with a sterile blade prior to placement of parasite laden bandage contact lens (BCL). The eyelids were closed by tarsorrhaphy with 4/0 silk sutures. The left eyes were the control eyes in which similar treatment was performed but receiving sterile SCL instead of parasite-laden BCL. No antibiotics, either topical or systemic, were used. Sutures were removed 4-5 days after initial tarsorrhaphy, and the SCL were removed. The animals will be euthanized and the corneas will be cut into two equal pieces. One piece will be homogenized in 1 ml of Page's saline using a glass tissue grinder and the homogenate be layered onto a lawn of E. coli, incubated and observed daily for appearance of "amoeba trails". The other piece will be processed for histopathology. Results: The animals were examined 4 days after the surgery. All the contact lenses were lost and some of the palpebral sutures were torn. None of the animals developed amoebic corneal infection. Conclusions: We have failed to reproduce the pig animal model of Acanthamoeba keratitis. However, there are many errors in the methodology that should be improved. We intend to repeat the experiment with total tarsorrhaphy including the suture of the third lid of the animal.