Effects of homeopathic medicines on macrophage – *E. cuniculi* interaction *in vitro*

Mirian Y.D.O. Nagai¹, Luciane C. Dalboni¹, Thayná N. Cardoso¹, Michelle S. Correia¹, Renata R. Palombo¹, Sandra A.G. Pinto¹, Cideli P. Coelho, Maria A. Lallo¹, Leoni V. Bonamin*¹*

¹Universidade Paulista, São Paulo, Brazil
* Corresponding author: leonibonamin@gmail.com

**Abstract**

*Encephalitozoon cuniculi* (*E. cuniculi*) is a fungus that behaves as an intracellular parasite infecting different types of cells. In rabbits and immunosuppressed animals of other species, including humans, it parasites neural tissues causing a highly characteristic neurological syndrome, for which repertory analysis indicates *Phosphorus*. Successful treatment of 7 naturally infected rabbits with *Phosphorus* confirmed that this medicine probably is the remedy of the ‘epidemic genius’ of microsporidiosis. In the present study, an *in vitro* model was used to evidence the intracellular relationship between macrophages and *E. cuniculi* after treatment of co-cultures with different potencies of *Phosphorus*. RAW 254.7 macrophages were co-cultivated with *E. cuniculi* and treated with *Phosphorus* 6cH, 30cH and 200cH. Controls were untreated co-cultures and cultures treated with vehicle (0.06% final alcohol concentration). Phagocytosis and lysosome activity were after after 1 and 24 hours of incubation by means of the calcofluor and acridine orange staining methods, followed by automatic image analysis (Metamorph®). Cytokine production was assessed with the MAGPIX®-Luminex system. The vehicle increased IL-6, MCP-1 and MIP1 production (p<0.05) and reduced the number of phagocytized parasites (p<0.0001) after 1-hour incubation, but no effect was detected after 24 hours of treatment. *Phosphorus* 6cH increased lysosome activity after 1- and 24-hour incubation and reduced the number of phagocytized parasites after 24 hours (p<0.05). *Phosphorus* 30cH increased lysosome activity after 1-hour incubation, followed by reduction of parasite internalization (p<0.001), and increased MCP-1 production (p<0.05) after 24 hours compared to both controls. The 200cH potency increased lysosome activity at 1 and 24 hours (p<0.05), together with reduction of internalized parasites (p<0.01) and increase of MCP-1 (p<0.05) compared to both untreated cells and cells treated with vehicle. The ultrastructural features of internalized parasites following treatment with *Phosphorus* 200cH is still under investigation. The results show that treatment of co-cultures with *Phosphorus* 200cH caused significant changes in the macrophage – *E. cuniculi* interaction, which might be involved in the clinical improvement of sick animals treated with this medicine.

**Keywords:** *Encephalitozoon cuniculi*, macrophages, *Phosphorus*, *in vitro* models

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