Co-culture with HepG2 spheroids spurs *in vitro* growth and development of the infective stages of the helminth pathogen *Fasciola hepatica*

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ABSTRACT

The helminth parasite *Fasciola hepatica* is a significant cause of animal and human morbidity worldwide. Part of the difficulty in developing new chemotherapeutics and vaccines for the control of Fasciolosis lies in our inability to culture and propagate juvenile worms *in vitro*. Several laboratories maintain *F. hepatica* short-term in simple media, but these are usually for the purpose of collecting excretory/secretory (E/S) products containing molecules important in parasite host interaction, rather than for biological studies. Here we show that the infective stage of the parasite, the newly excysted juvenile (NEJ), exhibit significant growth and development *in vitro* when co-cultured with spheroids derived from HepG2 cells, a human non-tumorigenic liver cell line with high proliferation rates and epithelial-like morphology. We investigated parasite development using antibody probes against two major NEJ proteases, FhCL1 and FhCL3, and by scanning electron microscopy (SEM). Parasites grown in the presence of HepG2 spheroids exhibit not only a rapid increase in size (length and width) but also extensive development of the gut caecum, musculature, and surface sensory system. Parasites were observed regularly interacting with the spheroids, sometimes invading the tissue, and moving between or tangentially to them indicating the importance of tactile stimuli. There was also evidence of parasites 'grazing' on the peripheral cells of the spheroids. We propose that the methodology developed here mimic *in vivo* parasite host liver interactions, greatly improving our ability to investigate and understand *F. hepatica*-host biology with future prospects for the development of new parasite control methods, such as vaccines and anthelmintic drugs.