

***Cryptosporidium* growth and development in monocultures – applications and limits**

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Cryptosporidium spp. infections have a significant effect on both human and animal health globally. Cryptosporidiosis is one of the leading causes of diarrhoeal mortality in children under five. *C. parvum* infections in ruminants can result in economic losses for farmers. In the UK and EU there are currently no drugs approved for use against *Cryptosporidium* spp., while Nitazoxanide is currently the only FDA approved in the US for treatment of infection in humans. Development of anti-parasitic drugs for *C. parvum* has been, in part, slowed by the inability to readily co-culture *C. parvum in vitro* with monocultures for extended periods of time, with HCT-8 cells typically supporting *C. parvum* growth for only 72 hours. Fertilisation in HCT-8 culture is currently the rate limiting step. While other systems, such as organoids, facilitate oocyst formation they remain inefficient. Here, we show the COLO-680N cell line readily supports prolonged growth of *C. parvum* beyond 72 hours. Using techniques such as qPCR, manual counting and fluorescence microscopy to validate prolonged growth and life cycle stage development. We present imaging of discrete lifecycle stages of *C. parvum* growth, complemented by fold-change analysis of key mRNAs associated with different life cycle stages *in vitro* which are significantly expressed during certain time points post infection. We have also used the COLO-680N system to determine GC₅₀ concentrations of two key drugs: Paromomycin (9.72µM) and Nitazoxanide (15.4µM) for *C. parvum*. Furthermore, we have been able to cryopreserve *C. parvum*-infected COLO-680N cells, with renewed growth validated using EdU-labelling. This technology enabling straightforward cryopreservation of clinical strains and transgenic lines. Overall, we track *C. parvum* development in COLO-680N cells over time, screen inhibitory compounds using this system and demonstrate straight forward methods for cryopreservation of *Cryptosporidium* from culture.