A droplet digital PCR (ddPCR) workflow for the detection of helminth and snail host eDNA in water and soil

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All organisms shed DNA into their surrounding environment (eDNA). Recently eDNA has been used for the detection of parasite species and has presented benefits over traditional sampling methodologies. The liver fluke, *Fasciola hepatica*, exhibits a complex lifecycle involving an aquatic snail intermediate host, commonly *Galba truncatula* at temperate latitudes, and a diverse array of mammalian definitive hosts, including agricultural ruminants and humans. Recently, there has also been growing interest in the rumen fluke species, *Calicophoron daubneyi*, in UK agriculture. The external life stages of rumen fluke complete a lifecycle almost superimposable to that of liver fluke and have been shown to use the same intermediate snail host. Despite the importance of the external environment in the fluke lifecycle, traditional diagnostic methods rely on the detection of infection either directly or indirectly from the definitive host.

Recent reports of eDNA isolation of both trematode parasites and their intermediate snail hosts have focused on environmental water samples. Despite *G. truncatula* spending considerable periods of time on mud surrounding water bodies, to date the presence of snail or parasite eDNA has not been examined in soil. The use of fully quantitative droplet digital PCR (ddPCR) platforms provides the opportunity for greater sensitivity and reproducibility of eDNA detection. To investigate the use of ddPCR for the detection of parasite and intermediate snail host eDNA, a workflow was developed utilising environmental samples (water and soil) collected from sheep and cattle farms in Northern Ireland. Environmental sample collection methodology and DNA extraction was optimised to allow reliable examination of parasite and intermediate host eDNA.

Analysis suggests that it is possible to detect parasite and snail intermediate host eDNA from environmental water and soil samples. The isolation of parasite and/or snail eDNA on a farm may provide pre-emptive warning of host infections earlier than current diagnostics, prompting further investigations and guiding anthelmintic treatment strategies.