t Can dogs become infected without eating parasitised mollusc intermediate hosts?

Abstract / Lay summary

Angiostrongylus vasorum, also known as the French heartworm or canine lungworm, is a parasite of growing importance, particularly in the fox and domestic dog. This nematode spreads via a molluscan intermediate host, such as the slug or snail. It has long been established that infection occurs when the molluscan intermediate host is eaten by the canine definitive host. This study examines a potential alternative route of spread via the mucus secreted by the intermediate host. At present, the study has progressed to the molecular stage where polymerase chain reactions (PCR) were performed on DNA extracted from A. vasorum-exposed slugs to identify slug species, the presence of nematodes and the presence of A. vasorum within the slug. This information will then be used, along with parallel slug digest examination results (where artificially digested slug tissue was examined for L3 larvae), to establish which slugs (either wild- µ P Z š (Œ E } u ‰ Œ E •] š Z Z } š • ‰ } š • [} Œ E - Z } o • ‰ } infected with Angiostrongylus) are (i) most likely to have released parasite larvae in their mucus when housed in the laboratory; and (ii) worthy of histological examination to establish the location of Angiostrongylus larvae within the slug host.

Introduction: aims and objectives

The aim of this study was to use the PCR technique to detect specific sequences of slug and parasite DNA. These results would then be used to establish which slugs were infected with A. vasorum only and which were infected with more than one species of nematode. This, in turn, would determine whether a general histological stain for fluorescent in situ hybridization should be used to establish the location of A. vasorum L3 within slug histological sections.

Methods and materials

DNA was extracted from slug tissue that had been collected during the first phase of this study. These slugs fell into three categories: control (A. vasorum cold spot), hot spot and A. vasorum-exposed slugs. Slug tissue digestion had already been performed on the A. vasorum-exposed slugs, so the infection status of each of those slugs was known prior to the start of the current study. In Phase 1 of the study, the researchers did not succeed in extracting DNA from L3 that had been released by digestion of A. vasorum-infected tissue. So one of the first goals of this study was to adapt the larval extraction technique so that DNA could be successfully extracted from L3. PCR was performed on extracted DNA using the three primer sets, 16S for slug identification and NC and ITS for nematode detection and identification

Results

The molecular part of this study was not completed due to a PCR contamination issue during the early phase of the project. However, the L3 DNA extraction protocol was successfully optimized so that we were able to amplify parasite DNA using nematode-specific primers. Also, there were slug tissues that were *A. vasorum*-negative using PCR, even though *A. vasorum* larvae had previously been released by digestion of tissue from those slugs. Fig.1 is an example of one such case. PCR using NC primers (for the detection of nematodes including *Angiostrongylus*) generated ~300 bp and 600 bp amplicons, the latter using DNA extracted from L3 (that morphologically appeared to be *A. vasorum*) released by tissue digestion of the same slug. This confirmed that this slug had been therefore infected with more than one species of nematode (polymorphic amplicon size).

Fig.1: Example of a nematode PCR result. Small amounts of DNA amplified via the PCR, then PCR products were E ^ Œ µ v _ through an agarose gel by electrophoresis, so that DNA of different sizes could be sorted. Key: Lanes 1 and 13, molecular weight ladders; lanes 2-11, amplicons from single nematodes recovered from small tissue samples from individual slugs; lane 12, amplicon from *Angiostrongylus* L3 recovered from a slug by peptic digestion.

Conclusion and discussion

We successfully completed PCR amplification from *Angiostrongylus vasorum* DNA extracted from L3 larvae recovered from slug tissue (Fig. 1). This was particularly important because many of the slug tissue NC PCR results that presented negative for nematodes were slugs that released larvae upon digestion. Thus, when studying *A. vasorum* in its intermediate host, PCR may not be a sensitive enough test to use when following the slug tissue protocol adopted here. This is probably because DNA was extracted from only 25 mg of slug tissue at a time, whereas peptic digestion was performed on half the slug. Homogenizing whole slugs prior to DNA extraction from