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

Abstract / Lay summary

Angiostrongylus vasorumalso known as the French heartworm or canine lungworm, is a parasite of growing importance particularly in the fox and domestic dothis nematode spreads via a molluscan intermediate host, such as the slug smail. It has long been established infection occurs when the molluscan intermediate host is eaten by the intermediate host. The study examines a potential alternative route of spread via the mucuse creted by the intermediate host At present, the study has progressed to the molecular stage here polymerase chain reaction (PCR) were performed on DNA extracted from A. vasorum exposed slug to identify slug species the presence of nematodes and the presence of A. vasorum within the slug. This information will then be sed, along with parallel slug digest examination results (where tificially digested slug tissue was examined L3 larvae), to establish which slug (either wild $\mu PZ \S (CE) u \% CE \S ZZ \S • \% \S • [CE - Z \S o infected with Angiostrongylus are (i) most likely to have released parasite larvae in their mucus when housed in the laboratory; and (ii) worthy of istological examination to establish the location of Angiostrongylus arvae within the slug host.$

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As it is commonly known that

A. vasoruminfections occur when canids consumatevasoruminfected molluscs, owners are advised to prevent their dogs from eating slugs smails However if molluscan mucus is found to be another route of infection, another area of concern in disease control will be brought to light.

Introduction: aims and objectives

The aim of this studywasto usethe PCR technique to detect specific sequences of slug and parasite DNA. These results would then be used to establish which slugs were infected withvasorumonly and which were infected with more than one species of nematod in turn, would determine whether a general histological stain theoretical in situ hybridization should be used to establish the location of A. vasorum within slug histological sections.

Methods and materials

DNA was extracted from slug tissue that had been collectering the first phase of this study. These slugs fell into three categories: control.(vasorumcold spot), hot spotandA. vasorumexposedslugs. Slug tissue digestion and already been performed on the vasorumexposed slugs, so the infection status of each of those slugs was known priorithe start of the current studyln Phase 1 of the study, the researchers id not succeed in extracting NA from L3 that had been released by digestion of vasoruminfected 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 PCR was performed on extracted DNA using the threeprimer sets, 16S for slug identification and NC and NO Amematode detection and identification

Results

The moleculapart of this study was not completed due to a PCR contamination during the early phase of the project However, the L3 DNA extraction protocol was successfully optimized so that we were able to amplify parasiteDNA using nematod specific primers. Also, there were slug tissues that were A. vasorum egative using PCR, even though vasorum arvae had previously been released by digestion of tissue from those slug ig.1 is an example of one such case CRusing NC primers for the detection of nematod including Angiostrongy lus generated ~300 band 600 bpamplicors, the latter using DNA extracted from L3 (that morphologically appeared to Abevasorum) released by tissue digestion of the same slugh is confirmed that this lughad been therefore infected with more than one species of nematod polymorphic amplicon size)

Fig.1: Example of a nematode PCR result. Small amounts of weak Amplified via the PCR, then PCR produces Λ ΔΕ μν through an agarose gety electrophoresis, so that DNA of different sizes ld be sorted. Key: Lanes 1 and 13, molecular weight ladders; lanes-21, amplicons from single nematodes recovered from small tissue samples from individual slugs; lane 12, amplicon from Angiostrongylus 3 recovered from a slug by peptic digestion.

Conclusion and discussion

We successfull completed PCR amplification fro Amgiostrongylus vasorum NAextracted from L3 larvae recovered from slug tissu (Eig. 1). This was particularly important because many the slug tissue NC PCR results that esented negative for nematods were slugs that eleased larvae upon digestion. Thus when studying A. vasorum 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 NAwas extracted from only 25 mg of slug tissue at a time, whereastic digestion was performed on half the slug. Homogenizing whole slugs prior to DNA extraction from