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Al-Sabi, Mohammad Nafi Solaiman; Webster, Pia; Willesen, Jacob; Davidson, Rebecca; Kapel, Christian

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DEPARTMENT OF AGRICULTURE AND ECOLOGY
FACULTY OF LIFE SCIENCES
UNIVERSITY OF COPENHAGEN

PCR Detection of *Angiostrongylus vasorum* in Canines

Mohammad Al-Sabi[1], Pia Webster[2], Jacob Willesen[3], Peter Deplazes[4], Rebecca Davidson[5], Christian Kapel[1]

[1]Department of Agriculture and Ecology, University of Copenhagen, Denmark, [2]Department of Disease Biology, University of Copenhagen, Denmark, [3]Small animal hospital, University of Copenhagen, Denmark, [4]Institute of Parasitology, University of Zurich, Switzerland, [5]Section for Wildlife Diseases, National Veterinary Institute, Oslo, Norway

Abstract

Angiostrongylus vasorum is spreading in foxes and dogs of northern Europe, therefore specific diagnosis is crucial for detecting the infection and assessing the disease prevalence. In this study, faecal samples were screened with a new PCR. Isolation of larvae by sieving facilitated the processing of larger sample volumes and allowed the recovery of dead L_1 from frozen samples. By the sieve-PCR method, a single larva per 2 g of faeces was detected and DNA of other carnivore helminths was not amplified, thus presenting a non-invasive tool for wildlife surveillance and for confirmative diagnosis in dogs.

Background and objectives

Carnivore angiostrongylosis is routinely diagnosed by morphological identification of L_1 recovered from faeces[1]. For safety reasons, faeces are frozen at -80°C to kill eggs of *Echinococcus multilocularis,* thereby excluding the use of the Baermann technique. Also copro-DNA extraction kits can process sample volumes of 0.2 g, which clearly limits the detection sensitivity. In this study, a new PCR method was designed and tested in an epidemiological study. A methodology of copro-DNA isolation from sieved larvae is also suggested.

L_1 of *A. vasorum* [1]

PCR design and application

Primers were designed from the ITS2 region of the rDNA with expected product size of 218 bp (Fig. 1). The detection limit of the PCR was tested with DNA from a single larva. The specificity of the PCR was confirmed when DNA of other carnivore helminths (3 trematode species, 5 cestode species and 11 nematode species, including *Crenosoma vulpis, Aelurostrongylus abstrusus*, and *A. falciformis*) was not amplified.

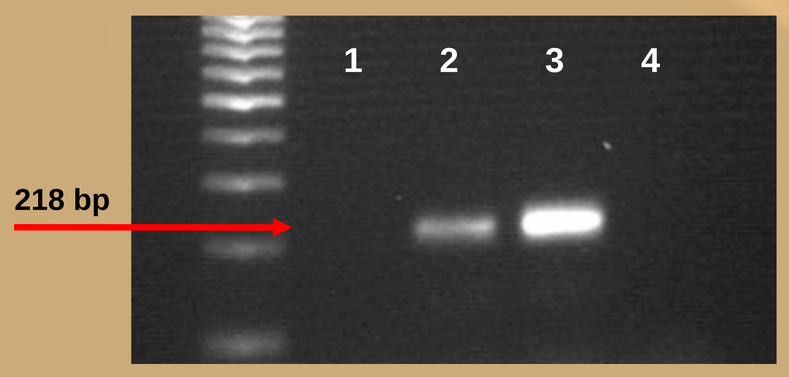

Fig 1. Detection limit and specificity of the new PCR. Lane 1: *Dipylidium caninum*. Lane 2: one L_1 of *A. vasorum*. Lane 3: five L_1 of *A. vasorum*. Lane 4: negative control.

Screening hunting dogs

The PCR was applied on freshly collected faecal samples from hunting dogs from Denmark (n=181) after larval concentration by Baermann method. Isolated L_1 of *A. vasorum* from three dogs were confirmed by PCR. This is the first record of an infected dog from the area of Jutland that previously visited the endemic area of Zealand; thus indicating the importance of dog transportation in the transmission of *A. vasorum* to non-endemic areas.

Screening foxes by copro-PCR of sieved larvae

The PCR was validated with L_1 from foxes naturally infected with *A. vasorum* (n=22, diagnosed by *post mortem*). Faecal samples from foxes free of *A. vasorum* infection (n=10) were used as negative control. Frozen larvae were isolated by sieving[2] followed by total DNA extraction[3]. By the sieve-PCR method, 2 g of faeces is processed, allowing the detection of 18 out of 22 infected foxes. Intermittent excretion of L1 is well documented and examination of multiple samples is recommended[1].

Applications and future trend

The new PCR can be used as a confirmative test in areas where *A. vasorum* is not endemic, hence precise morphological diagnosis may be hampered by the inexperience of the investigator. Also this PCR may have value as a confirmative diagnostic tool in wildlife, in travelling domestic dogs or in exotic animals. Finally, this PCR may potentially be used for larval identification in intermediate hosts, once its specificity can be tested with DNA of other relevant nematodes.

References

1. Koch and Willesen (2009) Vet J. 179: 348-359. 2. Mathis, Deplazes and Eckert, (1996) J Helminthol, 70: 219-222. 3. Al-Sabi, Kapel, Deplazes and Mathis, (2007) Parasitol Res, 101:731-736.

Notes

This project is accepted for publication in *Parasitology Research*. The background of this poster is a picture of the bursa of an adult male *A. vasorum* visualized by scanning electron microscopy[1].