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Characterization of *Enterobius vermicularis* in a human population, employing a molecular-based method from adhesive tape samples

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ABSTRACT

Human infection with the parasitic nematode *Enterobius vermicularis* occurs worldwide, particularly in children. Although its prevalence may exceed 35% in some parts of the world, molecular studies of *E. vermicularis* in humans are limited. The aim of the present study was to investigate the genetic variation within *E. vermicularis* in a human population. For this purpose, 77 adhesive tape samples taken from Greek children infested with *E. vermicularis* were tested. New primers were designed to amplify a segment of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene of *E. vermicularis* from adhesive tape samples. Thirty-six amplicons were sequenced and eleven different haplotypes were identified. All sequences clustered within the type previously characterized (type B), only reported to date from captive chimpanzees. To the best of our knowledge, this is the first study of *E. vermicularis* genotypes from a human population.

1. Introduction

Oxyuriasis, caused by the parasitic nematode *Enterobius vermicularis* (pinworm), is the most common helminth infection in Western Europe and North America [1]. The infestation occurs worldwide, affecting all socioeconomic groups, particularly school- and preschool-aged children. The estimated prevalence of *E. vermicularis* in children in various studies has been reported to be as high as 39% in Thailand [2], 37% in Sweden [3] and 29% in Denmark [4]. The infective helminth eggs reach the mouth or nose by direct contact with infected fingers or surfaces and are then ingested or inhaled (and subsequently swallowed). Alternatively, they can be transmitted indirectly, via contaminated food or fomites, brought to the mouth [5].

Diagnosis is traditionally made by the application of transparent adhesive tape to the perianal area, which is then examined microscopically for eggs [6]. Many animals are also parasitized by pinworms, most likely not transmissible to humans and vice versa. The only known exception is the chimpanzee (*Pan troglodytes*) that has been found to harbor *E. vermicularis* [7].

Genotyping studies of parasitic helminths are increasing nowadays, providing valuable insight into the geographic dispersal and diversity of helminths, their host range, specificity and helminth-host coevolution [8] and allowing monitoring of possible genetic restructuring of parasite populations, in the face of selection pressure from factors such as drug administration [9].

A very limited number of studies have been published to date on the molecular characterization of *E. vermicularis* [10,11]. Paleo-parasitological studies have investigated the presence of *E. vermicularis* in ancient coprolites and the sequence similarity between prehistoric and modern helminths [12,13]. Furthermore, the mitochondrial genome of *E. vermicularis* was reported recently [14]. A previous study, involving a DNA analysis of pinworms mostly in captive chimpanzees as well as a limited number of human samples, identified three major genotypic clusters, named types A, B and C [15].

Epidemiological studies based on molecular typing of *E. vermicularis* in humans have not been reported. Therefore, data on the existence of different genotypes in human populations and their geographical distribution has not been published. In fact, the scarcity of molecular data is quite surprising, considering that *E. vermicularis* is most likely a helminth that affects humans most frequently in industrialized countries [16].

The aim of the present study was to investigate the existence and distribution of different *E. vermicularis* genotypes in a Greek

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population infested by the parasite. New primers were designed to amplify a segment of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene of *E. vermicularis* DNA, directly from adhesive tape samples containing parasite eggs.

2. Materials and methods

2.1. Study population

Seventy seven adhesive tape samples from various urban and rural areas, in 5 Greek prefectures were examined (Fig. 1). The areas of sample origin and the number of samples examined are shown in Figs. 1 and 3. Infestation with the parasite was confirmed by microscopic examination of the adhesive tape preparations for *E. vermicularis* ova. Following the microscopic detection of the ova, the slides were stored at room temperature until DNA extraction.

2.2. DNA extraction

DNA was extracted from the samples using the QIAamp DNA Mini kit (Qiagen), according to the manufacturer's instructions, with the following modifications: a random piece of tape of 1–2 cm^2 was excised from each slide, immersed in ATL buffer containing 20 μL of proteinase K, and then incubated at 56 °C for 24 h. Following the addition of AL buffer, the sample was incubated for an additional 10 min at 70 °C. DNA was eluted in 100 μl of AE buffer.

2.3. PCR

Published primer sets to the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene [17] and to the internal transcribed spacer 2 (ITS-2) region of nuclear ribosomal DNA (rDNA) [18] were initially tested. A small number of positives were obtained using the first set and no amplification was observed with the second pair. New sets of primers were designed for a nested PCR, targeting sequences of the mitochondrial *cox1* gene, using the Primer3 program [19]. Primer sequences are as follows: for the outer PCR, EVM1 5′-TTTTTGGTCATCCTGAGGTTTATATTC-3′ EVM2 5′-CCACATTATCCAAAATAGGATTAGCC-3′ with an expected product of 390 bp, based on the mitochondrial genome sequence of *E. vermicularis* GenBank accession number (EU281143). For the inner PCR EVIF: 5′-TTGGTCATCCTGAGGTTTATATTC-3′ EVIR 5′-TCCAAAATAGGATTAGCCAACA-3′ with an expected product of 379 bp, also based on the aforementioned GenBank sequence. The inner primers were designed to overlap with external primers, in order to retain as much genetic information as possible. The EVIF primer is only 3 bases shorter than the EVM1 primer, so the reaction can also be considered semi-nested.

The first PCR was performed in a 50 μl volume, containing 1 × PCR buffer (Promega), 1.5 mM $MgCl_2$ (Promega), dNTPs (Fermentas) 0.2 mM each, 0.5 pmol of each primer (VBC Biotech), 1.5U *Taq* DNA polymerase (Promega), and 5 μL of the sample DNA. Thermal cycling was carried out in a Robocycler (Stratagene), as follows: an initial denaturation step at 94 °C for 5 min was followed by 45 cycles of 94 °C for 1 min, 57 °C for 1 min and 72 °C for 1 min, and a final elongation step at 72 °C for 10 min. Two μl of the outer

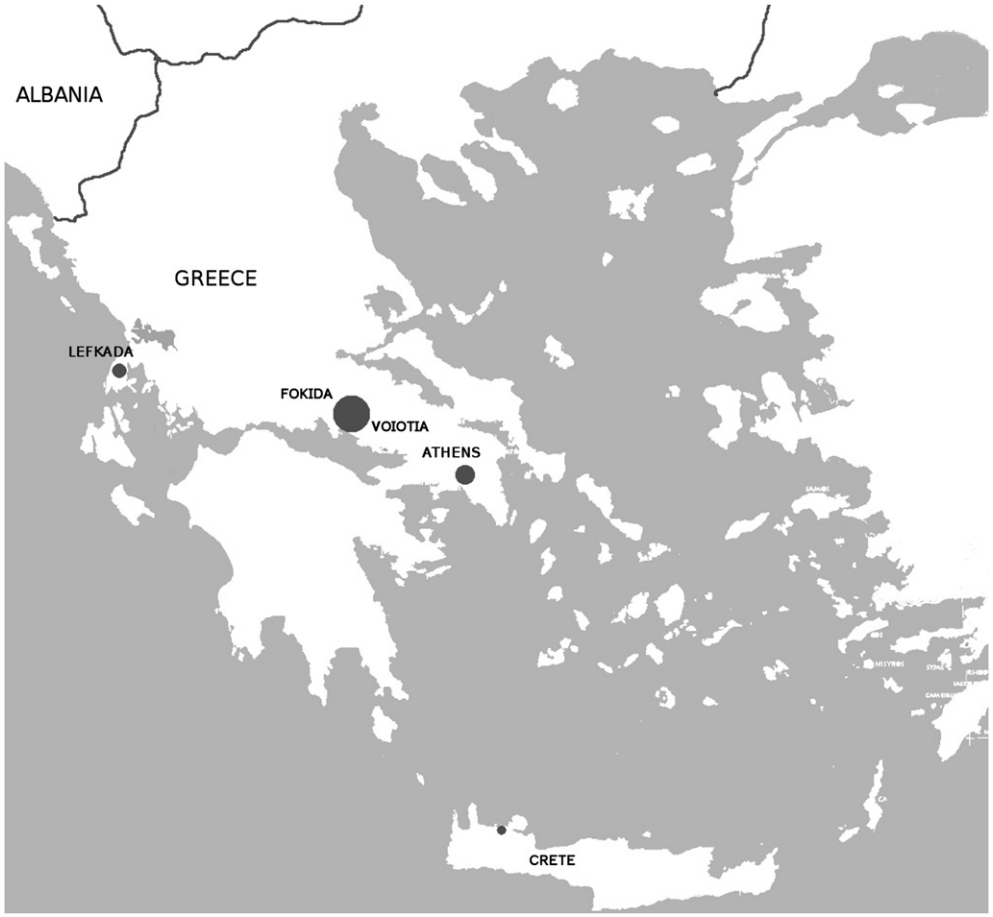

Fig. 1. Map showing the 5 Greek prefectures, where samples were collected. The size of the dots is roughly proportional to the number of samples examined.