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A high resolution melting real time PCR for mapping of filaria infection in domestic cats living in brugian filariosis-endemic areas

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ABSTRACT

We present here a real time PCR with high resolution melting (HRM) analysis for determining the prevalence and distribution of filarial species in domestic cats residing in brugian filariosis endemic areas of Narathiwat province, Thailand. Filarial species can be clearly distinguished in a single well using a single pair of primers. Blood samples were taken from a total of 2039 domestic cats living in endemic areas. Microfilariae were detected in 5.7% of the sample, while the overall prevalence of filaria infection by HRM analysis was 6.6%. The filariae species found in the infected cats were *Brugia malayi*, *Dirofilaria immitis*, *D. repens* as well as *Acanthocheilonema* (*Dipetalonema*) *reconditum*. This is the first report of *A. reconditum* infection from Thailand. The study also observed an overlapping of the distribution areas of animal and human filariae. From a public health perspective, the distribution and prevalence of these nematodes warrant an appropriate drug-based prophylaxis to be administered to cats in the endemic areas to reduce the number of diseased carriers. Furthermore, this molecular approach is more sensitive than microfilariae detection, enables species identification and greatly facilitates the collection of epidemiological data. Thus, the present study may help to bridge human–animal interface by coordinating research outcomes with the control of zoonoses that is vitally important for human and veterinary public health.

1. Introduction

In Thailand, endemic areas of brugian filariosis, caused by nocturnally subperiodic *Brugia malayi*, are located in the southern part of the country (Zielke et al., 1993). Several wild and domestic animals in endemic areas of Thailand, Indonesia, Malaysia, and Pacific island have been reported to be naturally infected with subperiodic *B. malayi* (Mak et al., 1982; Mak, 1984; Zielke et al., 1993). In domestic cats from Indonesia, two studies recorded infectivity rates at 7% (Masbar et al., 1981) and 6.1% (Palmeiri et al., 1985) while in Peninsular of Malaysia, the infectivity rate was 6.9% (Mak et al., 1980).

A survey of 66 domestic cats and 98 stray dogs in brugian filariosis endemic area in Chumphon province, southern Thailand, *B. malayi-like* microfilariae were observed in one cat while *Dirofilaria repens* and *D. immitis* were found in 2 cats and 34 dogs, respectively (Guptavanij et al., 1971a).

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Further south in Narathiwat province, a blood sample from 93 cats showed the presence of *B. malayi*-like mf with an infectivity rate of 4.3% (Guptavanij et al., 1971b).

Brugian filariosis in Narathiwat province appears to be a particular problem of the eastern part of the province which includes Mueang, Bacho, Ra-ngae, Su-ngai Kolok, Su-ngai Padi, Tak Bai and Joh Ai Rong Districts. It is speculated that the prevalence of brugian filariosis may be due to the presence of a large peat-swamp forest (Phru Toh Daeng) encompassing approximately 303 km^2 (Chaipattana Network, 1996) which may serve as a breeding place for *Mansonia*, the main vector of the nocturnally subperiodic *B. malayi* (Sucharit et al., 1975). The majority of the households in the endemic areas prefer cats as a family pet, thereby facilitating a ready opportunity for filarial transmission with humans due to co-habitation. Human infectivity with filariae of animals, referred to as zoonotic filariasis, occurs worldwide. Since the first report of zoonotic filariasis in the modern literature occurred more than 100 years ago (Babes, 1880; Addario, 1885), the numbers of cases and parasitic species has steadily increased (Orihel and Eberhard, 1998).

This emphasizes the urgent need to focus on these reservoir/infective hosts. However, the current state of knowledge about the filarial species, their prevalence and distribution in domestic cats living in acknowledged endemic areas is obviously limited due to the marked morphological similarities of these parasites (Harbut et al., 1995). In the case of *B. malayi* and *B. pahangi*, it is extremely difficult to differentiate the microfilaria of filarial species by Giemsa staining (Yen and Mak, 1978).

Diagnosis is essential in the management of disease, both at the level of individual animals and at the level of disease control in populations. Either microscopic detection or serodiagnosis that are widely used for diagnostic purposes, confer little guarantee of species identification in areas where both the human and animal filarial species coexist. The application of molecular technology could have a marked impact in distinguishing closely related species or subspecies.

We present here a real time PCR with high resolution melting (HRM) analysis for the screening of the status of endemicity in the acknowledged endemic areas. Filarial species can be clearly distinguished in a single well using a single pair of primers. Moreover, a large number of cat samples can be rapidly performed in a single run. This method offers the advantage of detecting at least 5 species of the filarial parasites in a single step PCR, which, in turn, supports the continued prophylactic treatment of the infected cats as well as the current state of knowledge about the extent and frequency of occurrence of the filarial species in domestic cats staying in recognized endemic areas.

2. Materials and methods

2.1. Animal ethical approval

The study protocol was approved by the Animal Ethics Committee of Faculty of Medicine Siriraj Hospital, Mahidol University, based on the Ethics of Animal Experimentation of the National Research Council of Thailand. The Certificate of Approval number is 004/2555.

2.2. Source of positive and negative control

The microfilariae (mf) of *B. malayi*, *B. pahangi*, *D. immitis*, *D. repens* and *Acanthocheilonema* (*Dipetalonema*) *reconditum* whose species were confirmed by the acid phosphatase staining method and HRM analysis, were used as positive controls. A parasite-free cat blood sample served as the negative control.

2.3. Study areas

This study surveyed filarial infection in the occasional feral cat and mostly domestic cats residing in households located in the brugian filariosis endemic areas of Narathiwat province. This data was obtained from the Filariasis Project, Pikhunthong Royal Development Study Center, Narathiwart province, Thailand. A total of 2039 domestic cats were randomly selected from 44 villages surrounding the central peat swamp forest, located in 4 districts, i.e. Su-ngai Kolok, Su-ngai Padi, Tak Bai and Mueng.

2.4. Blood samples

Using an ear-pricking procedure, blood samples were collected from the study population. Three thick blood smear slides were prepared from each cat. One slide was used in Giemsa staining, a second slide was used for DNA extraction and the last slide was used as a spare. The individual characteristics of each cat, including name, owner's name and address, date of extraction, gender and age were cataloged and entered into a standard record form.

Study samples consisted of stray and domestic cats from 8 sub-districts; i.e. 377 from Kaluwor Nuea, 458 from Kaluwor, 73 from Phraiwan, 293 from Bang Khuntong, 218 from Kosit, 310 from Sugnai Padi, 205 from Puyo and 105 from Prasemat.

2.5. Microscopic detection of microfilariae in the study samples using Giemsa staining

Giemsa staining was performed according to the standard WHO procedure (World Health Organization, 1991). Briefly, the dehaemoglobinized thick blood smear slides were immersed in freshly prepared working Giemsa stain for 45–60 min. Then, it was removed and rinsed by dipping 3–4 times in the Giemsa buffer. After air-drying, the slides were examined under a microscope (40×) for the detection of mf.

2.6. Extraction of filarial DNA from thick blood smear slides

Filarial DNA was extracted from the samples as follows: 100 μL of Tris–EDTA buffer solution was added onto a dehaemoglobinized thick blood smear slide and left for 5 min. Then the blood was scraped off the smear, transferred into a 1.5 mL microcentrifuge tube, and was centrifuged for 10 min at 15,520 × *g*. The Tris–EDTA buffer