ABSTRACT

The main objectives of this thesis are two pronged which include the development of a novel diagnostic technique and the creation a geospatial database for soil-transmitted helminthiasis in Malaysia. These aims have been successfully accomplished and the outcomes are detailed out in Chapters 5 and 7. Nonetheless in order to achieve these good outcomes, prior work in getting appropriate samples for high resolution melting (HRM) analysis and current data for geographical information system (GIS) had to be conducted in some epidemiological studies in the human and animal populations and these are illustrated in Chapters 3, 4 and 6. Therefore, this abstract will summarize our main findings relevant to the two main objectives.

In the present study, we have successfully developed a cost-effective and practical diagnostic tool based on real-time PCR coupled with HRM analysis which can serve as a reliable alternative technique for rapid and accurate identification of the five major hookworm species, namely *Necator americanus*, *Ancylostoma duodenale*, *Ancylostoma ceylanicum*, *Ancylostoma caninum* and *Ancylostoma braziliense* that are known to be endemic in Malaysia. These five hookworm species can be easily distinguished from one another based on their unique and distinct melting characteristic HRM curves. Each species has their own curve profiles and characterized by different melting temperature ($T_m$) peaks (i.e., *N. americanus*: $79.24 \pm 0.05^\circ C$ and $83.00 \pm 0.04^\circ C$; *A. duodenale* $79.12 \pm 0.10^\circ C$; *A. ceylanicum*: $79.40 \pm 0.10^\circ C$; *A. caninum*: $79.63 \pm 0.05^\circ C$; *A. braziliense*: $79.70 \pm 0.14^\circ C$). The present real-time PCR-HRM assay (100%) also has higher sensitivity compared to conventional semi-nested PCR (84.1%). However both techniques gave similar sensitivity (100%). The establishment of this new alternative diagnostic tool in this study has allowed accurate determination of
parasite prevalence, increased the understanding of transmission dynamics and provided important information particularly on the zoonotic potential of hookworm in high risk communities (i.e., Orang Asli communities). In these socioeconomically disadvantaged communities, the poor levels of hygiene and overcrowding, along with a lack of veterinary attention and zoonotic awareness coupled with uncontrolled population of stray and semi-domesticated dogs or cats exist in close proximity with humans, exacerbating the risk of zoonotic transmission. Our findings shown that *N. americanus* (89.6%) was the most common hookworm species found in human fecal samples, followed by *A. ceylanicum* (19.0%) while no *A. duodenale* infection was detected in this study. This result indicated that almost a quarter of hookworm-positive individuals are infected with *A. ceylanicum*, a zoonotic hookworm species that utilizes dogs and cats as definitive hosts. In fact, our study among dogs and cats inhabiting the same locations showed that hookworm infections (61.9%) were significantly higher in both animals compared to other parasite species. Of these hookworm-positive samples, 47.1% of the infected dogs and cats were also harboring *A. ceylanicum*. It is especially evident in these poor and deprived communities as uncontrolled populations of dogs and cats co-exist with the Orang Asli communities as demonstrated in our earlier epidemiological analysis which showed that close contact with dogs and cats were significantly associated with hookworm infections in the Orang Asli communities. Furthermore, our analysis via molecular tool on these *A. ceylanicum* positive isolates indicated that some of the *A. ceylanicum* strains from both human and animal host within the same geographical location were clustered together within the same group based on phylogenetic analysis. This provided evidence to show that dogs and humans may share genetically similar genotypes of *A. ceylanicum* within the same geographical location. Thus, the present study highlighted that it is essential to recognize that accurate
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diagnosis through practical, sensitive and specific analytical tools are crucial factors to address key epidemiology and population genetic questions to support surveillance, treatment and control program of STH infections.

Besides advancement and improvement in diagnostic tools, it is also vital to understand the STH biology and epidemiology with regards to their environmental and ecological limits. Significantly, we have demonstrated how the utilization of GIS coupled with remote sensing (RS) technology can play an important role in providing basic information for the implementation of sustainable and effective control programs for STH infections. We believe that findings of this study will have direct beneficial implications to STH control programs in Malaysia. This is particularly relevant that an accurate estimation of the total disease burden has not been fully explained although STH infections in Malaysia are still highly prevalent especially in rural and remote communities such as the Orang Asli. Moreover, these data are rarely available in the public domain in a form that is accessible to policy maker or relevant authorities for control program intervention. The current study has successfully utilized GIS to collate and map the geographical distribution of STH infections from available empirical survey data in peninsular Malaysia and highlighted areas where such information is lacking. The current assembled database represents one of the largest and comprehensive survey coalitions of STH infections in the country, consisting 99 survey locations conducted between 1970 and 2012 through our combined search strategies. As illustrated in our prevalence maps, the geographical distribution of STH vary considerably with no clear pattern across the surveyed locations. Given that only few survey data is available for most regions in Malaysia as illustrated in our prevalence maps, thus it is important to generate predictive risk map of infection on the basis of their ecological limits such as climate and other environmental factors. In the present
study, our correlation analysis between infection patterns and ecological factors indicated that Land Surface Temperature (LST) and Normalized Difference Vegetation Index (NDVI) were significantly associated with the observed prevalence of *A. lumbricoides*. On the basis of these two significant variables, we have managed to extrapolate predictive risk map of *A. lumbricoides* particularly in areas for which no data are available. The predictive risk map can serve as a baseline data in the estimated number of population at risk, numbers requiring treatment and cost of delivering anthelminthic at broad scale by prioritizing areas and populations at greatest needs. Our *A. lumbricoides* predictive risk map showed that prevalence of infection is low along the west coast and southern part of the country, whilst the prevalence is high along the central plain and northern part. Based on our estimation according to the latest available national census in 2010, it is estimated that up to 3.5 million individuals would be infected with *A. lumbricoides*. Of these, we estimated that there are 587,482 school-aged children in 75 out of 81 districts corresponding to 359 out of 842 sub-districts in the country who warrant mass drug administration (MDA) treatment at least twice a year according to WHO recommendations. Thus, this study reinforces that the utilization of GIS and RS techniques can provide the way forward in developing and implementing sustainable national control programs at realistic scale by identifying key endemic areas, providing more accurate estimates of populations at risk and basic information on treatment intervention by reducing the costs involved at both upstream (e.g., survey and design) and downstream (e.g., targeting, monitoring and evaluation) particularly when the recourses for control program are limited.