

CHAPTER 8
GENERAL DISCUSSION

The main objectives of this thesis were to develop a novel diagnostic technique and create 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**. However in order to achieve these good outcomes, prior work in getting appropriate samples for HRM and current data for 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 chapter on general discussion will summarize and link up all our main findings pertinent to the two main objectives.

Significantly in the present study, we have successfully developed a cost-effective and practical diagnostic tool based on real-time PCR coupled with high resolution melting (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. This assay has also proven to be more sensitive (i.e., 100%) and specific (i.e., 100%) compared to previous conventional semi-nested PCR (reference to chapter). Moreover, its inability to produce any amplification of control DNA representing a wide range of other intestinal parasites (i.e., nematodes and protozoa) signify its potential as an alternative diagnostic tool to other probe-based genotyping assays. The present study also showed that this technique can easily distinguish different hookworm species from various sample types such as samples obtained from different hosts (i.e., human vs. animals), sources (i.e., feces vs. adult worm) and life cycle stages (i.e., eggs vs. adult

worm). The results demonstrated that similar melting curves and profiles were produced regardless of whether the sources of the genomic DNA were derived from adult worms or eggs found in human or animal feces.

To the best of our knowledge, this is the first study to evaluate the potential use of real-time PCR coupled with HRM analysis for the detection and speciation of hookworm by employing the second internal transcribed spacer (ITS-2) of nuclear ribosomal DNA as a genetic marker in human sample. Since the introduction of HRM analysis technique, it has been widely used in various human clinical studies such as in mutation scanning, genotyping and sequence matching (Gundry et al, 2003; Wittwer et al, 2003; Liew et al, 2004; 2006; Zhou et al, 2004; Chou et al, 2005; Dobrowolski et al, 2005; Palais et al, 2005; Willmore-Payne et al, 2005; 2006; Cheng et al, 2006; Prathomtanapong et al, 2009; Radvansky et al, 2010; Saitsu et al, 2010). As for infectious diseases, it has been used in both microbial and bacterial studies (Erali et al, 2006; Slinger et al, 2007; Lilliebridge et al, 2011) and also in parasitological investigations of several parasitic protozoa (Bienz et al, 2001; Nicolas et al, 2002; Mangold et al, 2005; Robinson et al, 2006; Andriantsoanirina et al, 2009; Hussein et al, 2009; Pangasa et al, 2009; Nasereddin & Jaffe, 2010; Talmi-Frank et al, 2010) and parasitic helminths (Areekit et al, 2009; Radvansky et al, 2011).

As the current developed assay offers several advantages, it can potentially be used as an alternative tool in molecular epidemiology study for rapid screening of large numbers of samples especially for close related species of medical importance in a clinical laboratory. This is particularly relevant, for example in parasitic studies in which parasitic zoonoses pose a continuing public health problem in areas where human and animal species are co-endemic such as in developing countries where majority of populations live in poor and socio-economically disadvantaged conditions along with

close contact with domestic animals and poor veterinary care that put them at high risk of acquiring zoonotic infections. As shown in our study, almost quarter (19.0%) of hookworm-positive individuals are infected with *Ancylostoma ceylanicum*, a zoonotic hookworm species that utilize dogs and cats as definitive hosts (i.e., those studied and mentioned in **Chapter 5**). In fact, *A. ceylanicum* was the second most common species found in these communities after the anthroponotic human hookworm (i.e., *Necator americanus*). Likewise, 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* (i.e., those studied and mentioned in **Chapter 4** and **Chapter 6**).

This finding clearly indicated that *A. ceylanicum* may be more common than previously thought and this implied that dogs and cats may act as possible sources of infection to humans. It is especially evident in these poor and deprived communities as uncontrolled populations of dogs and cats co-exist with the Orang Asli communities. This finding was further strengthened by our earlier epidemiological analysis (i.e., those studied and mentioned in **Chapter 3**) which demonstrated that close contact with dogs and cats were significantly associated with hookworm infections in the Orang Asli communities. Moreover, our further analysis via molecular tool provided additional evidence of zoonotic exchange of *A. ceylanicum* between humans, dogs and cats in these communities indicating 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 provides evidence to show that dogs and humans may share genetically similar genotypes of *A. ceylanicum* within the same geographical location, a hypothesis that requires further investigation given the

small number of *A. ceylanicum* isolates (i.e., those studied and mentioned in **Chapter 6**). Thus, the present study highlighted that it is essential to recognize that accurate 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. In the current study, we have demonstrated how the utilization of GIS coupled with RS technology can play an important role in providing basic information for the implementation of sustainable and effective control programs for STH infections (i.e., those studied and mentioned in **Chapter 7**). We believe that findings of this study will have direct beneficial implications to STH control program in Malaysia. In recent years, geographic information system (GIS) has been widely used to map and analyze epidemiological distribution diseases and relate it to the factors that may influence their distribution patterns such as metrological and ecological factors (Brooker & Michael, 2000). The collection of these environmental data has been greatly enhanced by the used of remote sensing (RS) satellite data that can give proxy to environmental information (Hay, 2000). To date, GIS and RS approach have been increasingly used as important means to design and implement practical and low-cost control program of various parasitic diseases (Noma et al, 2000; Sabesan et al, 2000; Malone et al, 2001; Gyapong et al, 2002; Kabatereine et al, 2004; Clements et al, 2006; Hay & Snow, 2006; Diggle et al, 2007; Hay et al, 2007; Cecchi et al, 2009; Zoure et al, 2011) including STH infections (Brooker & Michael, 2000; Brooker et al, 2000; 2002a; 2002b; 2003; 2004, 2009; Knopp et al, 2008; Pullan et al, 2011; Tchuem Tchuente et al, 2012).

Although STH infections in Malaysia are still highly prevalent especially in rural and remote communities such as Orang Asli (i.e., those studied and mentioned in **Chapter 3**), an accurate estimation of the total disease burden has not been fully explained. Even though many surveys have been conducted especially in the endemic areas, 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. In an attempt to address this paucity, we used GIS to collate and map the epidemiology and distribution of STH infections from any available surveys in Malaysia. 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. Of this, almost all of our data (82.8%) were derived from published scientific academic journal, indicating the importance of this means in providing policy maker and public health authority greater access to the data. The distribution maps of STH infections as illustrated in this study presents the most detailed data currently available on geographical distribution of STH infections in Malaysia. Although there were many areas in the country where data are currently not available, the current collated database can assist in identifying and pinpointing where such information is lacking and assist in obtaining any additional data. The present map also provides the most current and detailed overall picture of the distribution of STH infections in Malaysia.

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 (Brooker & Michael, 2000). The ability to obtain the ecological variables has been further enhanced by the remote sensing (RS) technology that gives proxy to the

environmental factors and relates such factors with the prevalence data to determine which ecological factors may correlate with the transmission patterns (Hay, 2000). Besides, it also helps to improve our knowledge on STH in terms of their biology and epidemiology. In the present study, the environmental data, namely Land Surface Temperature (LST), Normalized Difference Vegetation Index (NDVI) and Digital Elevation Model (DEM) obtained from various sources were used to identify their ecological limits. Our logistic regression analysis indicated that Land Surface Temperature (LST) and Normalized Difference Vegetation Index (NDVI) were significantly associated with the observed prevalence of *A. lumbricoides*. In addition, our result also showed negative association between observed prevalence of *A. lumbricoides* with maximum LST and minimum NDVI. This means that when the temperature increases, transmission and prevalence of infection decreases. This illustrated that heat and low humidity (i.e., moisture) play important roles on the embryonation and survival of the STH ova or larvae, findings that were consistent with many studies on the thermal limits of STH either in field investigations (Dunn, 1972; Xu et al, 1995; Lai & His, 1996; Appleton & Gouws, 1996; Appleton et al, 1999; Hotez et al, 2003; Brooker et al, 2006), statistical analysis (Brooker et al, 2002a, 2002b; 2003; Pullan et al, 2011) or experimental and laboratory findings (Seamster, 1950; Anon, 1967; Beer, 1976).

On the basis of these two significant variables, we developed the predictive risk map of *A. lumbricoides*. The predictive risk map can provide basic information on the estimated number of population at risk, numbers requiring treatment and cost of delivering treatment at broad spatial scale particularly in areas without comprehensive data (Brooker et al, 2006). Likewise, it offers reliable indications of whether or not mass drug administration (MDA) intervention is warranted by prioritizing areas and

populations at greatest needs particularly when the recourses for control program are limited. 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 calculations 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 MDA treatment at least twice a year according to WHO recommendations (Anon,2002; 2005). Thus, our findings have shown that GIS and RS techniques can lead the way in assisting and developing sustainable national control program at realistic scale by identifying 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) (Brooker et al, 2006).