Dual Burden of Diseases in Resource Poor Countries: Diabetes Mellitus and Neglected Tropical Diseases

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Prof. Dr. Martin Spiess
Dekan

Dedicated to my beloved parents

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List of abbreviations

AIDS	Acquired immune deficiency syndrome
aaM	alternatively activated macrophages
ARBO	Arthropod-borne
ATM	Adipose tissue macrophage
CDs	Communicable diseases
CI	Confidence interval
CVD	Cardiovascular diseases
DALYs	Disability adjusted life years
DENV	Dengue virus
DBD	Dual burden of diseases
DF	Dengue fever
DHF	Dengue hemorrhagic fever
DM	Diabetes mellitus
DSS	Dengue shock syndrome
EPG	Egg per gram
LF	Lymphatic filariasis
GAD	Gutamic acid decarboxylase
HbA1c	Glycated hemoglobin A1c
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IDF	International diabetes federation
IL	Interleukin
LADA	Latent autoimmune DM in adults
LF	Lymphatic filariasis
MDA	Mass drug administration
MODY	Maturity onset of DM of young
NCDs	Non-communicable diseases
NOD	Non-obese diabetes
NTDs	Neglected tropical diseases
NLRs	NOD-like receptors
OR	Odds ratio
SD	Standard deviation
SES	Socio-economic status

STH	Soil-transmitted helminth
TLRs	Toll-like receptors
WHO	World Health Organization

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Summary

Background: The prevalence of diabetes mellitus and neglected tropical diseases are nowadays escalating, especially in endemic resource poor countries and their impact on social and economic development are negatively huge. Recent studies indicate that diabetes mellitus problem is now truly global epidemic and a number of risk factors are contributed to diabetes. Dengue viral infections have been one of the most reemerging diseases worldwide in recent years and case fatalities are usually high among dengue infected individuals having any underlying co-morbid disease condition. Helminth or parasite infections mainly contribute to global burden of diseases and cause adverse events on health. Therefore, it is very important to understand the interaction between diabetes mellitus and neglected tropical diseases in clinical management for curative, as well as disease epidemiology for preventive purposes.

Methods: Firstly, we conducted a systematic review and meta-analysis to summarize and update the current knowledge of diabetes mellitus and dengue viral infections as well as helminth or parasite infections. To follow up the findings between diabetes and parasite infections and to explore their association in epidemiological settings, we conducted two field studies. A cross-sectional community based study was carried out among adults in 4 provinces of Lao People's Democratic Republic. We also investigated a longitudinal study among schoolchildren in poor neighborhoods of South Africa to figure out the comorbid association and effect of helminth infections on blood sugar concentration determined by HbA1c measurements after deworming.

Results: In this PhD work, we found a positive relationship between diabetes mellitus and neglected tropical diseases we studied. Firstly, our systematic review and meta-analysis summarized that diabetic patients are more likely to have a severe form of dengue if they were infected with a dengue viral infection. In the context of relationship between diabetes mellitus and another neglected tropical disease, our findings showed that the prevalence and species of helminth infections were different in different country settings. In Laos PDR, food born trematodes were more prevalent and the prevalence of diabetes was also higher than estimated figures. We observed a positive relationship between diabetes mellitus and teania infection in Lao PDR setting. In South Africa, we found soil transmitted helminth infections and *H. pylori* infections were quite common among schoolchildren and the high numbers of prediabetic prevalence. A positive relationship between diabetes mellitus and *H. pylori* infections has been revealed in the study. No association has been found between diabetes and

other infections. We also disclosed that HbA1c levels are more likely to increase after deworming.

Conclusion: Our research work provides valuable insights of co-morbid association. It can be concluded that diabetes mellitus and dengue or taenia or *H. Pylori* infections have influenced each other not in a good way, either dengue infection or teania or *H. Pylori* infections exacerbate diabetes mellitus or vise visa. The patho-physiological mechanism behind these associations should be further explored. As diabetes is a multi-faceted disease, many other factors could also top up these interactions. Diabetes, dengue, parasitic infections remain of major public health concerns in both Laos PDR and South Africa. Hence, our findings call for action to establish a proper public health policy with an integrated approach addressing both diseases condition to reduce mortality and/or morbidity and to ameliorate clinical outcomes and preventive measures among affected population. Additional studies in other endemic countries are also greatly recommended to reflect our current findings to be able to apply for the implementation as a global perspective.

Chapter 1
Introduction

1.1 Dual burden of diseases (DBD)

For decades, communicable diseases (CDs) and chronic non-communicable diseases (NCDs) occurred principally in different areas; low- income countries were primarily burdened with infectious diseases such as diarrhea, respiratory infections, hepatitis, malaria, tuberculosis, NTDs, HIV/AIDS and so on, worsened by poverty. Chronic illnesses such as cardiovascular diseases, diabetes mellitus (DM), obesity, cancer, and asthma mostly affected the economically well-developed part of the world. Traditionally, in a very young age period, CD or infectious diseases such as diarrhea and acute respiratory infections, tuberculosis, measles and malaria, and nutritional related problems such as vitamin B_1 deficiency called Beri Beri, vitamin D deficiency and iron deficiency anemia, are very common. In adulthood, accidents and violence-related disabilities and deaths become a major concern while CD such as TB, HIV, malaria, NTDs still exist, and in older age groups, NCDs such as DM and cardiovascular diseases, followed by cancer and degenerative disorders are generally dominating. Due to socio-economic developments, globalization, lifestyle transition and longer life expectancy especially in low- and middle- income countries, these resource-poor countries are nowadays facing both kinds of problems called a dual burden of diseases (DBD); increasing NCD trends are superimposed to continuously high levels of existing, emerging and re-emerging CD (Marshall, 2004). This DBD creates a critical challenge to the health of people and to health systems. The World Health Organization (WHO) presented the proportional distribution of disability-adjusted life years of CDs, NCDs and accidents among low income and high income countries in 2002 and estimates for 2030. According to the figure, in 2002, CDs accounted 56% of all infections, NCDs for 33% and accidents for 11% and in 2030, it will be 41% for CDs, 45% and 14% for NCDs and accidents respectively. The figures show that low income countries would simultaneously have to deal with the problems of DBD in near future. In high income countries, the proportional distribution of disability adjusted life years (DALYs) for NCDs were 85%, 9% for CDs with 6% of accidents in 2002 and by 2030, it is predicted that they will particularly be suffering more from NCDs accountable for 90%, with only 10% for the rest (7% of CDs and 3% of accidents) (Mathers and Loncar, 2006).

An interrelation between chronic diseases and infections has also been evidenced, most importantly for diabetes mellitus (DM). DM puts patients at a higher risk for infections

that are common in low- and middle-income countries, such as for tuberculosis (Joshi et al., 1999) and possibly other infections including malaria (Ina Danquah et al., 2010). Uncontrolled DM furthermore predisposes to candidiasis, otitis externa and rhinocerebral mucomycosis, *Streptococcus* and *Klebsiella* infections due to suppressed immune response and increased susceptibility to the infections (Mueller, 2011). Whether infections such as tuberculosis or HIV directly increase the risk of DM is still poorly understood (Remais et al., 2013, Mueller, 2011). There is a growing evidence for an increased risk of type 2 DM in patients with chronic HCV infection (White et al., 2008), however, there are also other studies reporting type 2 DM as a predisposing factor for HCV infection (Guo et al., 2013) and we call it a two way association (Hammerstad et al., 2015). As recently summarized, a relevant part of cardiovascular diseases may be attributable to neglected tropical diseases (NTDs) and other infections prevalent in developing world (Moolani et al., 2012).

Understanding the link between CDs and NCDs is of great policy relevance in terms of estimating global burden of disease in a population-specific manner towards preparing specific health systems for the challenges ahead. The directions and mechanism underlying the interrelations are likely to vary greatly for specific diseases (Knapp, 2013). Current evidence for the biology that mediates the link between CDs and NCDs is still limited.

1.2 Diabetes Mellitus

DM is a chronic metabolic disease which is uniformly characterized by chronic hyperglycemia, which is the result, though, of different pathophysiological processes. DM has traditionally been subdivided into type 1 (autoimmune destruction of insulin-secreting β cells), type 2 DM (insulin resistance and features of metabolic syndrome) and gestational DM which develop high blood sugar levels only during pregnancy. Yet, this dichotomous classification captures the range of the disease poorly and in fact insufficiency of pancreatic β cells in producing adequate amounts of insulin as well as some degree of insulin resistance seem involved in all types of DM. DM can also be divided into subgroups characterized by a) destruction of β cells, b) defective response to glucose, c) low β cell mass from birth, and d) defective processing of insulin (Tuomi et al., 2014).

DM in children and adolescents or type 1 DM remains the most common form of DM in children younger than 15 years of age. Its incidence varies substantially between different populations (from 0.51 in China to 40.90 in Finland). The incidence has rapidly risen in recent decades globally. As a result of the increase in childhood obesity, type 2 DM has emerged as a new type of pediatric DM after age of 10 years, usually after the onset of puberty. Puberty is

normally associated with transient insulin resistance, which in healthy adolescents is balanced by an increase in insulin secretion. In obesity this increase is insufficient to overcome insulin resistance. The prevalence of type 2 DM in Asian adolescents is sometimes even higher than in US teenagers, such as in the case of Chinese teenagers. In fact, in both children and adults, African-American, Hispanic, Asian-Pacific-Islander and American-Indian persons seem to have higher incidence and prevalence of type 2 DM than non-Hispanic whites. But the ethnic differences are strongly dependent on lifestyle contexts. The diagnostic classification of children and adolescents into type 1 and type 2 DM is not straight forward as auto-antibodies and ketoacidosis have also been detected in a considerable percentage of children with type 2 DM (Tuomi et al., 2014).

DM in Adults or type 2 DM: While the age cut-off to differentiate between type 1 and type 2 DM was originally between 35-40 years, this cut-off is not a valid clinical value these days. At around age 20-50 years not only type 1 and type 2 DM but also maturity onset of DM of young (MODY) and secondary DM can occur. Obesity and metabolic syndrome have generally been used as the basis for diagnosis of type 2 DM but today people who develop type 1 DM are often obese, too. In fact, the diagnostic value of obesity and metabolic syndrome lies in their absence for diagnostic confirmation of non-type 2 DM. Latent autoimmune DM in adults (LADA) has been introduced as a subtype of DM in response to the fact that adult onset DM may need immediate initiation of insulin therapy and be associated with pancreatic autoantibodies. Whether LADA is a clinical subtype of DM or merely reflecting the stage of disease is not clear. Around 5-14% of adult DM patients have pancreatic antibodies to glutamic acid decarboxylase (GAD). Positivity for autoantibodies may be transient in both, children and adults. LADA is associated with a better cardiovascular risk profile (serum triglyceride, HDL cholesterol, obesity and hypertension) than type 2 DM, but whether this translates to fewer cardiovascular events has not been sufficiently studied. Other hybrid forms of DM exist. For example, African-Americans patients of sub-Saharan African descents and particularly obese men have a form of non-autoimmune ketosis-prone DM (Tuomi et al., 2014).

Genetic background: While monogenetic forms of DM exist (MODY) both type 1 and type 2 DM are polygenic diseases with over 60 susceptibility loci identified in the context of genome-wide association studies. Genetics has provided clear support for the view that LADA is between adult-onset type 1 DM and GAD-antibody-negative type 2 DM (Tuomi et al., 2014).

Gene – Environment Interactions: Asian populations seem to be supersensitive to risk factors for type 2 DM (Tuomi et al., 2014). Chinese people for example seem to be susceptible to even slight increases in body mass index (BMI); the mean BMI value at the moment of DM diagnosis in Chinese patients is 25.9 kg/m^2. In the American Nurses' Health Study patients of Asian ethnicity had about double the risk of type 2 DM for the same BMI compared to Caucasian patients (Pan et al., 2004). Asian persons have more abdominal adipose tissue for any given BMI than their non-Hispanic white counterparts. DM in Asian persons is characterized by a combination of both, insulin resistance and failing β cells. Lifestyle modification can result in substantial reductions in DM risk in these populations, such as in India or China (Tuomi et al., 2014).

1.2.1 Epidemiology

Globally, as of 2015, an estimated 415 million people have DM worldwide, with type 2 DM making up about 90% of the cases and three fourth of people with DM live in low and middle income countries (WHO, 2014). One in 11 adults had DM while one in two adults with DM is undiagnosed and one person in every 6 seconds dies from DM causing 5 million deaths in 2015. DM overall at least doubles the risk of premature death. By 2040, the number of people with DM is expected to rise to 642 million and one in 10 adults will have DM (IDF, 2015).If DM is not properly controlled or left untreated without being diagnosed, the people living with diabetes may have to deal with short-term or long-term complications. The short-term complications includes diabetic emergencies such as hypoglycemic diabetic ketoacidosis (DKA) or hyperosmolar non-ketotic acidosis (HONK) which requires immediate intensive treatment, otherwise, it causes coma or death. The main long-term complications includes affecting the eyes and blindness (retinopathy), heart (cardiovascular diseases), kidney failure (nephropathy), nerves (neuropathy) that lead to amputation of the limbs, miscarriage and still birth in the diabetic pregnant mothers.

Among 20 leading causes of YLDs at global level in 2011, DM ranked 6th which was not changed since 2000, representing 21.8 million. In terms of DALYs, DM ranked 12th in 2011, topping from 18th in 2000, representing 56.4 million (WHO, 2013). Despite the fact that DM reduces life expectancy, it is associated with substantially higher lifetime medical expenditures. The person with DM expects approximately 2.3 times higher medical expenditures than persons without diabetes and 12% of global health expenditure $673 billion is spent on DM in 2015 (IDF, 2015).

1.3 Neglected tropical diseases

NTDs feature a group of different type of infections caused by a variety of pathogens which are bacteria, viruses, protozoa and helminths. The WHO considers 17 neglected tropical diseases to be tackled namely Buruli ulcer, Chagas disease, dengue and Chikungunya, Dracunculiasis (guinea-worm disease), Echinococcosis, endemic treponematoses (Yaws), foodborne trematodiases, human African trypanosomiasis (sleeping sickness), Leishmaniasis, Leprosy (Hansen disease), Lymphatic filariasis, Onchocerciasis (river blindness), rabies, schistosomiasis, Soil-transmitted helminthiases, Taeniasis/Cysticercosis and Trachoma (9 are caused by microparasites and 8 are caused by macroparasites), affecting more than 1 billion people worldwide mostly in 149 endemic countries . They mainly affect populations living in poverty, without adequate sanitation and in close contact with infectious vectors and domestic animals and livestock. The figure 1 describes the numbers of NTDs currently presenting in endemic countries.

1.3.1 Epidemiology

In 2010, it was estimated that NTDs collectively caused 26 million Disability Adjusted Life Years (DALYs) worldwide. NTDs devastate human health and can cause death. Substantial efforts are underway on a global scale towards understanding the dual disease burden related to DM infectious diseases such as tuberculosis and HIV. In contrast little research has been conducted on the association between DM and specific neglected tropical diseases as defined by the WHO (Table 1) (Hotez et al., 2014), even though several NCDs, ranging from cancer to cardiovascular and liver disease result from chronic NTD infections (Hotez and Daar, 2008).

Biological evidence suggests that the association between DM and NTDs may well vary by type of infection, as evidenced with the example of helminthic infections versus dengue fever. Helminthic infections and dengue fever contribute substantially to the NTD associated global burden of disease (Hotez. et al., 2014), so that it is of public health relevance to study their association with DM. The examples of helminthic and dengue infections prove the complexity of the DM-infection association. In the case of Dengue fever, available evidence would suggest that DM mellitus may have an adverse effect on the clinical course of the infection. In the case of helminth infections, they may represent the other extreme of the spectrum wherein, the helminth mediated immune-modulatory effect can in fact dampen inflammation and can potentially confer protection against metabolic diseases (Van Riet et al., 2007).

Table 1.1 Neglected tropical diseases and estimated global burden (Hotez. et al., 2014)

Disease	**DALYs from GBD 2010 (numbers in parentheses indicate 95% CI)**
NTDs	26.06 (20.30-35.12)
Intestinal nematode infections	5.19 (2.98-8.81)
Hookworm disease	3.23(1.70-5.73)
Ascaris	1.32 (0.71-2.35)
Trichuriasis	0.64 (0.35-1.06)
Leishmaniasis	3.32 (2.18-4.90)
Schistosomiasis	3.31 (1.70-6.26)
Lymphatic filariasis	2.78 (1.8-4.00)
Food-borne trematodiases	1.88 (0.70-4.84)
Rabies	1.46 (0.85-2.66)
Dengue	0.83 (0.34-1.41)
African trypanosomiasis	0.56 (0.08-1.77)
Chagas disease	0.55 (0.27-1.05)
Cysticercosis	0.50 (0.38-0.66)
Onchocerciasis	0.49 (0.36-0.66)
Trachoma	0.33 (0.24-0.44)
Echinococcosis	0.14 (0.07-0.29)
Yellow fever	<0.001
Other NTDs*	4.72 (3.53-6.35)

*Relapsing fever, typhus fever, spotted fever, Q fever, other rickettsiases, other mosquito-borne viral fevers, unspecified arthropod-borne viral fever, arenaviral haemorrhagic fever, toxoplasmosis, unspecified protozoal disease, taeniasis, diphyllobothriasis and sparganosis, other cestode infections, dracunculiasis, trichinellosis, strongyloidiasis, enterobiasis, and other helminthiases.

1.4 Dengue viral infection

Dengue is one of the most important arthropod-borne (ARBO) tropical infection caused by the dengue virus, family *Flaviviridae*; genus *Flavivirus* with its four main serotypes (DEN-1, DEN-2, DEN-3 and DEN-4), transmitted by the *Aedes aegypti* mosquito.

1.4.1 Epidemiology

The number of dengue virus infections has increased 30-fold over the last decades. Dengue is an endemic disease in the tropics and subtropics and is found in the Caribbean, South and Central America, Mexico, Africa, the Pacific Islands, Southeast Asia, the Indian sub-continent, Hawaii, and Australia. Approximately 2.5 billion people, or 40% of the world's population, live in areas where there is a risk of dengue transmission (CDC, 2016). The number of cases reported increased from 2.2 million in 2010 to 3.2 million in 2015 (WHO, 2014c). An estimated 500,000 people with severe dengue require hospitalization each year and about 2.5% of those affected die (WHO, 2014c). Not only is the number of cases increasing as the disease spreads to new areas, but explosive outbreaks are occurring. In 2012, an outbreak of dengue occurred on the Madeira Islands of Portugal resulted in over 2,000 cases. Cases have occurred in Florida of United States of America, Yunnan province of China

and Laos in 2013. Worldwide large dengue outbreaks were recorded in the Philippines reporting more than 169,000 cases, Malaysia exceeding 111,000 suspected cases of dengue, representing a 59.5% and 16% increase in case numbers to the previous year, respectively. Brazil alone reported over 1.5 million cases in 2015, approximately 3 times higher than in 2014 and Delhi, India, also recorded its worst outbreak since 2006 with over 15,000 cases (CDC, 2016).

Dengue has long been viewed as a pediatric disease but the average age of dengue cases has been rising and recently adults to be at increased risk for dying from dengue infection. The increase in tourism in tropical regions also contributed to the increase in adults dengue cases (Tantawichien, 2012). WHO recognizes dengue virus as a major and emergent concern due both to its expanding distribution and also to an increased frequency of epidemics.

1.4.2 WHO classification of Dengue viral infection

In 1975, the dengue viral infection was developed as dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) case classification of dengue by experts based on studies on Thai children in the 1950s and 1960s. DF was defined as having the symptoms of dengue fever which begins three to fourteen days after being infected with dengue virus with a clinical presentation of high fever, headache, vomiting, muscle and joint pains. Dengue fever mostly resolves itself without any treatment; however, it can progress to its severe form which is called dengue hemorrhagic fever. DHF was characterized by fever lasting from 2-7 days with the evidence of hemorrhagic manifestation or a positive tourniquet test and decreased platelet counts (thrombocytopenia:≤100,000 cells per mm^3). In 1997, four grades of DHF were defined as DHF grade I, II, III, and IV), where Grades III and IV are clarified as DSS, with hypotensive shock or narrow pulse pressure plus clinical signs of shock. The revised WHO 2009 classification consists of dengue without warning Signs, dengue with warning Signs, and Severe dengue. Severe dengue is defined as dengue with any of the following: (1) severe plasma leakage leading to shock or respiratory distress, (2) severe hemorrhage, or (3) any organ failure (Figure 2).

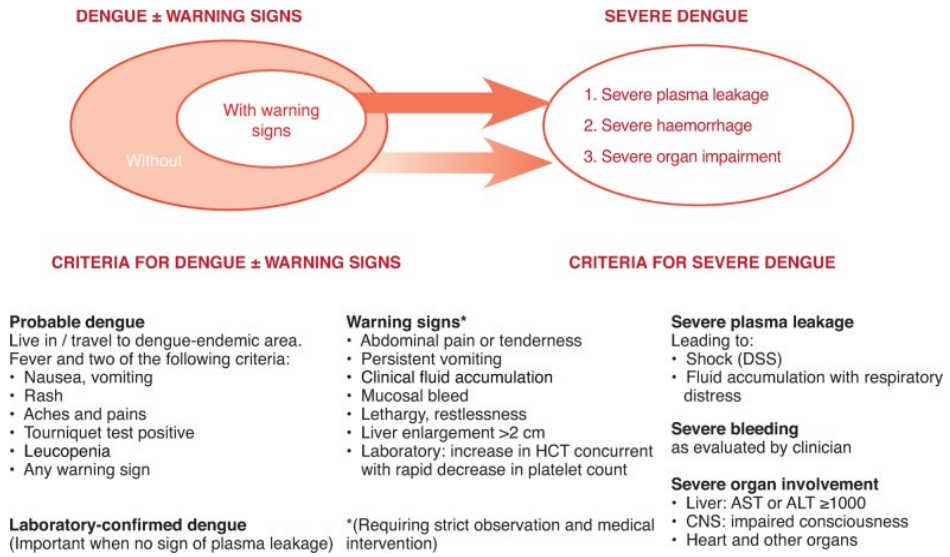

Figure 1.1 The revised WHO classification of dengue by 2009 (Hadinegoro, 2012)

The WHO classification (2009) defines DHF as a clinical entity with plasma leakage as the cardinal feature that differentiates it from DF; the 2009 classification lists several clinical manifestations as qualifiers for severe dengue. The revised WHO classification for severe dengue appears to have higher sensitivity and specificity to identify cases in need of heightened care (Narvaez et al., 2011).

1.4.3 Immunological association between dengue viral infection and DM

It has been assumed that patients with dengue disrupt microvascular endothelial cell function, possibly with the altered metabolic release of proinflammatory cytokines. The initial immune reaction of dengue infected cell is to produce a number of interferon and cytokines against viral infection through the defensive innate immune system. Although elevated levels of proinflammatory cytokines and chemokines occur in patients with DF, a surge in the numbers of monocyte chemo attractant protein-1 (MCP-1), vascular endothelial growth factor (VEGF), tumor necrosis factor (TNF)-α, IL-6, IL-8, IL-10, chemokine ligand (CCL)-2, CCL-3, CXCL-8, CXCL-10, and interferon (IFN)-γ have been reported in patients experiencing severe dengue disease (Rothman, 2011, Navarro-Sánchez et al., 2005, Chaturvedi et al., 2000, Bozza et al., 2008, Leong et al., 2007). They are mainly responsible for increasing endothelial permeability or vascular leak, which exacerbate third space fluid shifts characteristic of severe dengue (Limonta et al., 2008). Similarly, types 2 DM, a metabolic disorder, creates the physiological changes in integrity of the endothelium due to a chronic inflammatory condition caused by T- lymphocytes activation leading to release of MCP, TNF-α, INF- γ and VEGF (Brausewetter et al., 2001, Makino et al., 2005). All these cascaded cytokines which play a fundamental role in triggering intrinsic permeability of endothelial surface of the cell in both

diabetic and dengue infected condition increase the severity of dengue infection into severe form or death to DENV infected DM patients by third space plasma shift, hemoconcentration, hypotension and then, finally shock stage. Therefore, dengue patients with co-morbid DM require more medical care with longer duration of hospitalizations than dengue patients without diabetes (Mallhi et al., 2015, Pang et al., 2012). In clinical setting, dengue patients with diabetes have a tendency having thrombocytopenia (reduced platelet) counts, an indicator of the severity of vascular leakage in dengue infection and also a monitoring indicator for disease progression (Chen et al., 2015).

Present evidence suggests that viral factors and additional personnel characteristics as well as potentially DM might adversely influence the clinical course of an infection. The association between dengue and DM is of relevance in the light of the shift of dengue to older ages and the steep increase in the prevalence of DM. However, the evidence was never summarized systematically (Figueiredo et al., 2010b).

1.5 Helminth infections

Helminths also known as worms are living in and feeding on living hosts (humans and other animals). Depending on their morphology, helminths are classified into flatworms, tapeworms, roundworms and flukes. Most of them reside in the intestines but some in blood vessels.

1.5.1 Epidemiology

Many helminth infections are contributing to the disease burden of NTD: e.g. soil-transmitted helminths (STH) with 5.2 million DALYs; schistosomiasis with 3.3 million DALYs; lymphatic filariasis (LF) with 2.8 million DALYs; onchocerciasis with 0.5 million DALYs. Sub-Saharan Africa, America, China and Asia have the highest burden of NTD (Global Burden of Disease Study, 2014). Implications of this figures for the control of NTDs were discussed more recently in a review by Hotez and colleagues (Hotez. et al., 2014). Based on DALYs as well as years lived with disability, STH, i.e. roundworm (*Ascaris lumbricoides*), the whipworm (*Trichuris trichiura*) and hookworms (*Necator americanus* and *Ancylostoma duodenale*), affect the largest proportion of those affected. More than 1.5 billion people or 24% of the world's population are infected with one or several species of STH. The morbidity caused by these infections contributes to poverty, decreased productivity, and inadequate socioeconomic development (WHO, 2014). Over 270 million preschool-aged children and over 600 million school-aged children are currently living in areas where these parasites are highly prevalent, in some areas affecting the vast majority of the population.

Moreover, East, Southeast Asian countries as well as central and south America has high prevalence of foodborne trematode infections (flukes) due to ingestion of contaminated food or fish which are infected with larvae and at least 40 million people have trematode infection (WHO, 2014a).

1.6 Hygiene hypothesis

It has been hypothesised that differences in the prevalence of infections contribute importantly to the geographical variation of the prevalence of autoimmune diseases (Selmi, 2010). In 1989, the hygiene hypothesis was first formulated by Dr Strachan. He proposed that lack of exposure to infections in early childhood may protect against allergic diseases and that the increasing cleanliness of household environments would explain in part rises in allergic diseases as observed in the second half of the 20th century in Western Europe, North America and Australia (Bloomfield et al., 2006). The hygiene hypothesis was later expanded to explain more generally increased incidences of immune mediated diseases such as asthma, multiple sclerosis, inflammatory bowel diseases and autoimmune DM (Okada et al., 2010).

1.7 Factors that increase the allergies and autoimmune diseases

1.7.1 Geographical variation

The prevalence of autoimmune disorders shows substantial geographic variation with generally higher prevalence rates in affluent countries. The incidence of type 1 DM in Bulgaria or Romania was lower compared to Western Europe (Green et al., 2001), incidence of type 1 DM in Finland is six times higher than in adjacent Russia despite similar genetic background (Kondrashova et al., 2005). Similarly, the incidence of asthma was higher in the residents of former West Germany than East despite their similar genetic background (Bloomfield et al., 2006). A higher prevalence of multiple sclerosis is observed in northern Europe compared to southern part (Pimentel, 2013).

1.7.2 Migration

Migration accounts one of the factors for the rapid increase in the incidence of allergic and autoimmune diseases. Migration studies illustrated the increasing frequency of type 1 DM in Pakistani families moving to the United Kingdom (Staines et al., 1997) and Somali children who had moved to Finland as high as Finnish children (Tuomi et al., 2014). The risk of multiple sclerosis is also increasing in Asian immigrants moving to the United States of America (Okada et al., 2010) and in Israel, multiple sclerosis is common among immigrants from Europe compared to those from Africa or Asia or Israelis (Kurtzke, 2000).

1.7.3 Decreased infectious diseases

Infectious diseases in early years of life are declining due to better quality of health care and services, good public health measures, vaccination against common childhood infections and the wide use of antibiotics. For instance, infants who received antibiotics had a higher incidence of allergy and other atopic disorders than children who had not received antibiotics in their infantile period (Droste et al., 2000).

1.7.4 Socio-economic related factors

The epidemiological studies reveal that there is a correlation between the socio-economic status (SES) and autoimmune disorders; positive correlation between gross national product and incidence of asthma, type 1 DM and multiple sclerosis in 12 European countries (Bach, 2002), the low incidence of type 1 DM is correlated with low average socio-economic indexes such as unemployment, lack of a car, crowded housing conditions, and living in rental housing rather than purchased property in a study in Ireland (Patterson et al., 1996), Crohn's disease was also related with SES in the Canadian province of Manitoba (Blanchard et al., 2001) and privileged SES with higher incidence of atopic dermatitis in Hannover, Germany (Werner et al., 2002).

1.7.5 Environmental factors

A wide range of factors contribute to decreased early childhood infections in affluent regions include improved sanitation such as decontamination of the water supply, pasteurization and sterilization of milk and other food products, and cleaned living conditions. The PARSIFAL study and GABRIELA study showed that children living and growing up on farms with a range of microbial exposures largely explained the protective effect on the development of asthma in children than children in the reference groups (Ege et al., 2011).

1.7.6 Genetic factors

The genetic factors also play an important role in the pathophysiological mechanism of autoimmune disorders. The rate of atopic dermatitis is much higher among monozygotic twins (77%) than among dizygotic twins (15%) due to atopic dermatitis-specific genes (Bieber, 2008). The hygiene hypothesis proposed that human genes have adapted to the constant exposure of infectious agents results in immune imbalances. Compared to other microbial pathogens, genome-wide association studies showed that the effect of immunological reactions induced by helminths on IL genes has been stronger than for other infections (Fumagalli et al., 2009).

1.8 Chronic helminth infections and immunomodulation

Several lines of evidence suggest that geographic and temporal differences in the prevalence of helminth infections may contribute to the changes in autoimmune diseases over time and between regions (Van Riet et al., 2007). Despite their broadly different characteristics, most helminth infections evoke similar adaptive immune responses in their human host. Generally helminth infections induce a shift from Th1 to Th2 phenotype, accelerate T regulatory and B regulatory phenotypes, and attenuate the levels of the inflammatory cytokines (Figure 1) (Bashi et al., 2015, Van Riet et al., 2007). Immunomodulation is hypothesized to protect the helminth from being eradicated by the human organism and to protect the human host from organ damage caused by excessive pro-inflammatory responses. The immunomodulation is not only directed to helminths, but also to non-related antigens, which would explain the inverse association of chronic helminth infection with allergies, auto-immune diseases, and vaccine response.

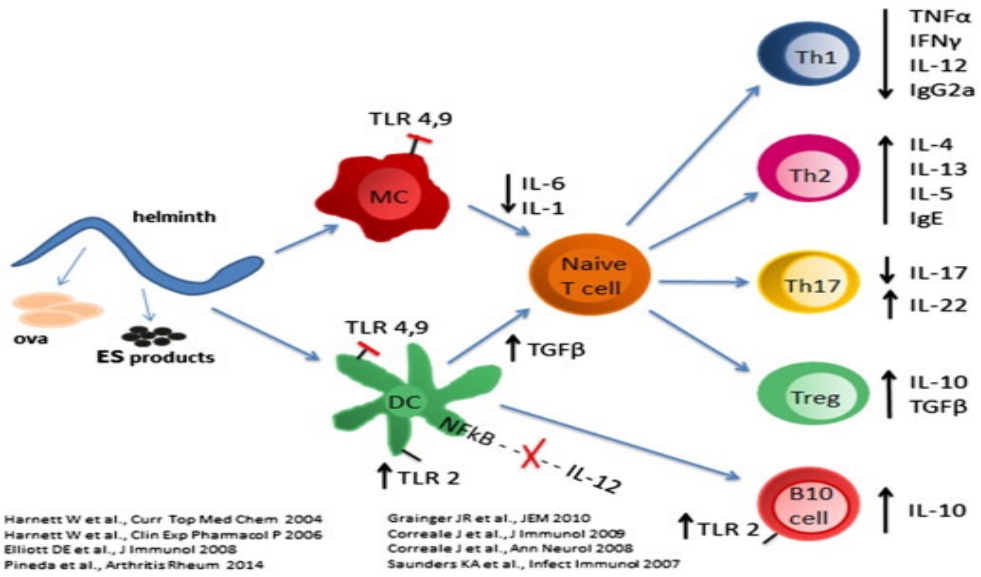

Figure 1.2: Mechanism behind helminthes immunomodulation in autoimmunity (Bashi et al., 2015)

1.8.1 Immunomodulation due to chronic helminth infections

Soil-transmitted helminthes (STH): Stronger Th2 cytokines were associated with lower burden of STH infection (Jackson et al., 2004). The children living in areas helminths hyperendemic area have higher level of IL-10 and TGF-ß especially due to intestinal nematodes *A. lumbricoides and T. trichiura* than the children living in mesoendemic areas in Cameron and Brazil (Figueiredo et al., 2010a, Turner et al., 2008). The higher production of

these cytokines or hyperesponsive effects to helminth probably helps the worms' survival in the human body. Chronic ascariasis and trichuriasis infection was associated with altered gene expression profiles with modified Th2 responses (i.e. up-regulation of IL-5 and IL-10), down regulation of neutrophil function and up-regulation of eosinophils (Reina Ortiz et al., 2011). In mice, chronic STH infection induced down-regulation of a single micro RNA which can abundantly be seen in alveoli of normal lungs but not in the lungs fibrosis (Pandit et al., 2010) indicating that STH affect not only the immune system but also target organs of the host (Reina Ortiz et al., 2011).

Schistosomiasis: Animal schistosomiasis studies revealed that Th1 cells mainly contribute to immunological mechanism, but Th2 responses are associated with resistance to reinfection of schistosomiasis (Dunne et al., 1992, Roberts et al., 1993). Pro-inflammatory responses with elevated level of TNF and IFN-γ were shown in Kenyan patients (Mwatha et al., 1998) and higher IL-13 in Brazilian studies (Alves Oliveira et al., 2006). In addition, Th17 cells are believed to be associated with schistosome induced pathology in both animal (Rutitzky et al., 2008) and humans models (Mbow et al., 2013). Down modulation of effector responses by IL-10 and TGF-β, and others also seem to be involved in the prevention of schistosomiasis (Baumgart et al., 2006, Herbert et al., 2004, Turner et al., 2011) and the involvement of Treg, IL-10 production B cells which could also be present in murine and in human chronic schistosomiasis (van der Vlugt et al., 2012). Recently, aaM (alternatively activated microphages) as a key player in controlling the immune pathology in schistosomiasis has also been highlighted (Barron and Wynn, 2011).

Lymphatic filariasis: Upregulation of Th1, Th17 cells and antibody responses are observed in filariasis infected patients (Babu et al., 2009). The studies have been revealed that immune there are increased production of natural Treg as well as IL-10 producing effector cells in the individuals infected with *W. bancrofti* or *Mansonella* (Metenou et al., 2010). In pathogenesis of onchocerciasis, the role of Th2 mediated Treg and IL-13 is significant and was related to IL-13 gene mutation which is responsible for allergic hyper-reactivity in the body (Hoerauf et al., 2002, Satoguina et al., 2002).

Yet it is important to keep in mind that helminth related immunomodulation is affected by several key elements such as the types of helminths species, intensity of the infection and the host's immune system. In most of the cases, helminths will induce tolerance, but in some scenarios, they may cause an inflammatory response. Affliction spectrum ranges from low pathology/tolerance, along with high parasite burden, to chronic disease/inflammation, along with low parasite infection intensity (Bashi et al., 2015).

1.8.2 Immunomodulation of chronic helminth infections in NCDs

Asthma and Allergy: Allergic diseases such as asthma, eczema and rhinitis are mainly type-2 mediated and helminth infections urge IL-10 or TGF-ß level leading to the development of allergic immune response. A study in Gabonese children, who were infected with Schistosomiasis, and another study among Vietnamese school children infected with hookworm showed that IL-10 production in response to the helminth antigens, was inversely associated with skin prick test (SPT) reactivity to house dust mite allergen (Yazdanbakhsh et al., 2002, Flohr et al., 2010). Similar findings have been reported in a study of Ecuadorian children with *A. lumbricoides* infection, (Cooper et al., 2008). There is also evidence that the IgE cross-reactivity between helminth Ags and allergens such as with cockroach (Santiago et al., 2012) or mite (Acevedo et al., 2009) might influence the outcome of allergy.

Multiple sclerosis: The MS patients who were infected with helminths demonstrated higher production of IL-10 compared to uninfected patients (Correale and Farez, 2007). In another immunological study, active colitis was linked to the cytokine-producing CD4 and IL-17 Th cells, and the presence of IL-22 Th cells in the colonic mucosa indicated helminth colonization and disease remission. The types of released cytokines also depend on the place where the infection is occurred e.g. higher levels of IL-4, IL-25, and RELMβ (Broadhurst et al., 2010) in helminth-occupied ascending colon; elevated IL-17 and TNF as well as reduced expression of RELMβ (Weng et al., 2007) in the rectum. In a murine model, bacterial-colitis with *Heligmosomoides polygyrus* was related to increased aaM (Weng et al., 2007). Another chemically induced-colitis model with *Hymenolepis diminuta* infection showed an expression of FIZZ1/RELMα and argininase-1 which attenuated colitis (Hunter et al., 2010).

Rheumatoid arthritis: The rodent studies have shown that helminth infections or helminth extracts can suppress or prevent arthritis (Matisz et al., 2011). A case-control study in India showed that no circulating microfilariae or filarial antigens was found in the patients with rheumatoid arthritis, in contrast to healthy controls (Panda et al., 2013).

Cardiovascular disease and atherosclerosis: A couple of risk factors contribute to cardiovascular disease including atherosclerosis which is due to the inflammatory process of lipids. Studies on the role of anti-inflammatory mechanism illustrated the role of Treg that lessened atherosclerosis (Ait-Oufella et al., 2006). One of the lipid lowering agents also has anti-inflammatory action. Thus, balancing the proinflammatory and inflammatory actions in the body is important in the endothelial function regulation. It is known that helminths disturbance the nutritional status of the host and thus turns into the beneficial effects by reducing the serum lipid levels (Stanley et al., 2009). Apolipoprotein deficient mice showed a

rapid development of atherosclerosis over a short period time (Stanley et al., 2009). Regards to the helminths, mice with *S. mansoni* was found about 50% less in the development of atherosclerotic lesions (Doenhoff et al., 2002) and soluble factors released from *S. mansoni* live eggs has an impact the cholesterol lowering effects. Another study in mice injected with schistosoma-frozen eggs showed reduced total cholesterol and LDL level compared to non-injected ones, but no difference in atherosclerotic formation (La Flamme et al., 2007). These studies suggest that helminth infections or related products are acting as lipid-lowering agents in the development of atherosclerotic formation process.

1.9 Immunomodulation due to chronic helminth infections: Potential role relevance to DM

The auto-destruction of β cells in the pancreas in type 1 DM and related forms (e.g. LADA) is mainly Th1 mediated. The Th2 shift associated with chronic helminth infection is thus hypothesized to decrease this Th1-related self-destruction of insulin producing cells.

The effect of helminth infections and helminth antigens on type 1 DM has been intensively studied in the type 1 DM non-obese diabetic mouse model. Observed alterations in the immune system in response to helminth antigens involved several types of immune cells including B cells, tolerogenic dendritic cells, aaM, granulocytes and innate lymphoid cells. They all induced Th2, NKT and Treg generation or expansion. The cytokine secretion evoked from NKT, Th2 and Tregs feeds back and maintains the tolerogenic and alternatively activated phenotypes of antigen presenting cells. As a result, diabetogenic (Th1) cells become regulated resulting in only a non-destructive insulitis (Zaccone and Cooke, 2013). In the case of infection with *L. sigmodontis*, transforming growth factor (TGF) β production was a crucial factor for DM prevention (Hubner et al., 2011). In another study, *N. brasiliensis* infection leads to accumulation of eosinophils and associated aaM, the predominant macrophage phenontypes in adipose tissue from lean mice. As a result, infected obese mice were better able to maintain glucose homeostasis (Wu et al., 2011).

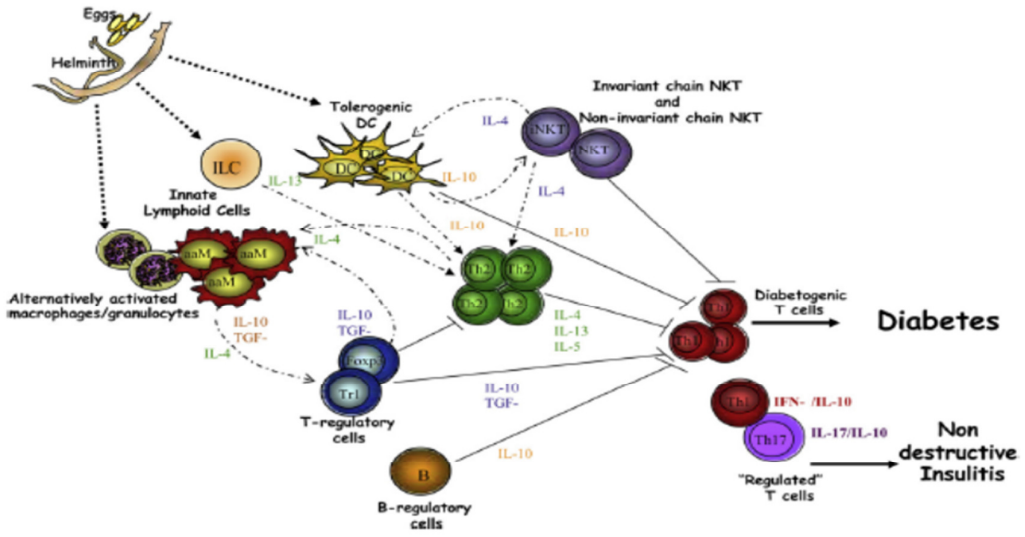

Figure 1.3 Mechanism of helminth antigen-mediated diabetes (Zaccone and Cooke, 2013)

Chronic helminth infections could additionally counterbalance sterile metabolic inflammation and thus insulin resistance by re-shifting the balance from Th1 to Th2 and Treg cells. The insulin resistance, a predominant characteristic of type 2 DM and related forms (e.g. LADA) is strongly linked to obesity and specifically to visceral fat mass and therefore to obesity-associated metabolic inflammation. This is a sterile inflammation produced in response to metabolic rather than infectious stimuli. Obesity related damage-associated molecular patterns, such as saturated fatty acids, can activate inflammatory signaling pathways in part via toll-like receptors (TLRs) and via NOD-like receptors (NLRs) and thereby lead to macrophage inflammation. In addition to receptor-mediated pathways, inflammatory signaling can be stimulated by cellular stresses such as reactive oxygen species (ROS), ER stress, hypoxia and lipotoxicity, which can all be enhanced in the obese insulin-resistant state. Metabolic inflammation mediates insulin resistance through the inhibition of insulin signaling. Inflammation can also affect insulin action indirectly by modulating various metabolic pathways, resulting in the production of "second messengers", such as fatty acids, that promote insulin resistance. Proinflammatory M2-type adipose tissue macrophages in obesity are critical effector cells in the initiation of inflammation and insulin resistance and therefore a key etiological mechanism linking the increasing incidence of type 2 DM and obesity. Obesity can also cause inflammation in tissue other the adipose tissue such as for example in pancreatic islets or liver. In addition to adipose tissue macrophage (ATM) alterations, obesity also alters the activity of T cells which are next to ATMs the largest immune cell component of adipose tissue. Th1 and Th17 cells are pro-inflammatory whereas

Th2 cells and T regulatory (Treg) cells are anti-inflammatory in the context of obesity. The number of Th1 cells increases in obesity, whereas the abundance of Th2 and Treg cells, which elicit immunosuppressive effects, decreases.

1.9 The effect of helminth infections in prevention and treatment of autoimmune diseases including DM

Epidemiological studies suggest that the helminth exposure might prevent or treat autoimmune mediated diseases or disorders. Anthelminthic therapy is currently being studied as a promising treatment for several (non-viral) autoimmune diseases including Crohn's disease(Hunter and McKay, 2004), asthma (Falcone and Pritchard, 2005) and ulcerative colitis (Saunders et al., 2007). 2500 ova *Trichuris suis* were administered to active Crohns disease patients to determine the possible efficacy and safety of intestinal helminthes as treatment option. The study demonstrated that 72% of patients had improved symptoms with no adverse reaction (Hunter and McKay, 2004). In another study, *T. suis* ova were given to patients with active ulcerative colitis and the study found significant improvement of symptoms (43% improvement *versus* 17% for placebo) after 6 weeks (Saunders et al., 2007). However, in allergic rhinitis, *T. suis* ova therapy had no effect even though it induced a substantial clinical and immunologic response (Bager et al., 2012). Hookworm (*N. americanus*) is also being investigated for therapeutic use. Two previous studies reported that infection with *N.* larvae was strongly associated with protection against asthma symptoms (Scrivener S, 2001) and in people with allergic rhinoconjunctivitis (Scrivener S, 2001). Inoculation of Crohn's disease patients with infective *N. americanus* larvae subcutaneously led to a slight improvement in disease symptoms (Croese et al., 2006).While hookworms have some pathogenic potential effect (Hotez et al., 2004) they are likely to be safe at low infection intensities (Pritchard et al., 1999).

Observational studies evidenced that intestinal parasites are associated with reduced risk and severity of allergy and other autoimmune diseases. A prospective cohort study in Argentina showed that multiple sclerosis patients with parasite infections (*H.nana*, *T. trichiura*, *A. lumbricoides*, *S.stercorais*, and *E.vermicularis*) showed a significantly lower number of exacerbations and disease disability scores compared to those without parasite infection. Immunological mechanisms involved included an increased production of IL-10 and TGF-beta, CD25+CD4+ FoxP3+ T cells, and induced regulatory T cells leading to a less severe disease course (Correale and Farez, 2007). People living in *S. mansoni* endemic area were found to suffer less from wheezing and use of anti-asthmatic drugs compared to the

persons living in non-endemic area (Araújo et al., 2004). In the context of DM, the influence of parasitic infections on the onset of autoimmune DM was studied experimentally in non-obese diabetic (NOD) murine models of T1DM. NOD mice were infected with *S. mansoni* (or exposed to *S. mansoni* cercarae or antigen positive) (Cooke, 2009), *T spiralis* (Saunders et al., 2007), *H.polygyrus* (Liu et al., 2010), *L. sigmodontis* (Hubner et al., 2011) and *D. immitis* (Imai et al., 2001). The helminth exposures were found to prevent the onset of type 1 through protection from Th1-mediated β cell destruction in this mouse model (Bashi et al., 2015). Children with a frequent intake of anthelminthic drugs against *A. lumbricoides* and *T. trichiura* exhibited a higher rate of positive skin prick test for house dust mite compared to the children not treated or not taking anthelminthic drugs (van den Biggelaar et al., 2004).

1.10 Relevance of the research to Laos People Democratic Republic and South Africa

Laos PDR, one of low middle income countries, is experiencing the DBD. The regional Asia and Pacific prevalence of DM between 20-79 years of adults was 9.3% with 153 million of DM people in 2015 and it is predicted to increase 215 million in 2040 (IDF, 2015). According to WHO Diabetes 2016 Country Profile for Lao PDR, DM prevalence is 5.6% in the general adult population. Approximately 2% of the Lao population dies due to DM annually, with a 3.6% prevalence rate among 20-79 years aged adults (WDF, 2017). Due to increasing DM prevalence, the Laotian government starts to emphasize the DM as an important health concern of the country.

The prevalence of DM in African populations has been increasing rapidly in recent decades and 20 million of people are living with DM (20-79 years), 29.7 million with IGT. The number of people with DM in the region are predicted to increase up to 41.5 million by 2035 (an increase of 50%) (IDF, 2015). South Africa is one of the top 5 countries in Africa for having high DM prevalence and according to WHO, the prevalence rate of DM in South Africa was estimated to be 9.2% and the number of diabetic cases was approximately 2.6 million (20-70 years of age) in 2014. The prevalence was even higher in a cross-sectional survey conducted by Peer et al. among recruited 1,099 participants, (392 men and 707 women) aged 25–74-year-old, black Africans in Cape Town. The aim of the study was to assess the prevalence of the diabetes and to compare the findings with 1990 study. The WHO 1985 diabetes criteria were used to diagnose the diabetes and the results were compared. The prevalence of age adjusted diabetes were 12.2% in 2008/09 compared to 8% in 1990, IGT: 11.7% in 2008/09 and 7% in 1990. Among diabetes cases, 57.9% were known cases and only 38.6% of them were treated. The study clearly showed that the prevalence of the diabetes has

been increasing over the decades and the numbers are expected to further increase in coming years (Peer et al., 2012).

As dengue fever belongs to the tropical and subtropical world as an important infectious disease, dengue infections or DHF mostly occur in urban and suburban areas in the Americas, South-East Asia, the Eastern Mediterranean, and western Pacific and in rural areas in Africa. Laos PDR is one of dengue endemic countries and dengue ranked on 4th place (8.1%) among top leading causes of death in 2012 (WHO, 2015). The country has suffered from the worst ever recorded outbreak and nearly 50,000 people have contracted dengue fever in 2013. 70% of the cases were children (OCHA, 2013). Dengue outbreaks are usually cyclical and occur every 3 to 5 years. In early 2017, 215 dengue cases have been reported and dengue serotypes change has recently been recorded in affected provinces (WHO, 2017). In Africa, the epidemiology of dengue is not clear even though *Aedes* spp. mosquitoes are extensively scattered and can serve as vectors of dengue virus in Africa. Besides, when the population of suspected dengue cases in Africa is compared with those of Asia or the Americas, the incidence of dengue cases are highly underreported because most febrile illness in Africa is usually assumed to be malaria due to low awareness of the health care providers and diagnosis capacity (Msimang et al., 2014). A study was conducted in South Africa aiming to describe the epidemiology of laboratory-tested dengue cases from 2008-2013 and the results showed the increasing trend of dengue infection over 5 years. The dengue detection rate in collected specimens is 6.1% (83 confirmed cases per 1364 dengue tests), 4% (9/249) in 2011, 6% (19/326) in 2012 and 14 % (21/146) in 2013 and most of the cases were among South African travelers returning from the dengue endemic countries (Msimang et al., 2014).

The intestinal parasitic infections do not usually get much attention by the health care decision makers like other bacterial or viral infections as most of the cases are subclinical. In Southeast Asia, especially Mekong region, food born trematodes and STHs remain important public health concern. An estimated 67.3 million people worldwide are mainly at risk for *Opisthorchis viverrini,* and about ten million infected people live in Thailand and Laos (Sithithaworn et al., 2012). A couple of helminth studies have been conducted in Laos in recent years and different prevalence of helminth infections have been reported across the country. In Campacsack province of Southern Laos, multiparasitism was very common and hookworm was the most prevalent STH species was hookworm (76.8%), followed by *A. lumbricoides* (31.7%) and *T. trichiura* (25.0%). *O. viverrini* (64.3%) and *S. mekongi* (24.2%) infections were also found in the study area (Sayasone et al., 2011). Moreover, Korea-Laos Collaborative Project for Control of foodborne trematode Infections surveyed among a total

of 6,178 residents and revealed the highest prevalence of O. veverrini with minute intestinal flukes (55.6%) followed by hookworms (27.8%) and *T. trichiura* (6.5%) in middle and southern area of the country (Savannakhet, Khammouane, Vientiane (Nam Ngum), Champasack (Khong Island), and Saravane province). The survey also reported of *A. lumbricoides* (33.8%), hookworms (47.8%), and *T. trichiura* (32.6%) were observed in middle and northern area of the country namely Phongsaly, Luang Prabang, and Vientiane (Nam Ngum) areas, respectively (Eom et al., 2014). In Laos, consumption of raw and insufficiently cooked fish is a main risk factor for high prevalence of food born trematodes. Other risk factors such as drinking contaminated water, improper hand washing and practicing poor body and environmental hygienic conditions are also contributing to the infections.

The studies in South Africa have showed high prevalence of intestinal nematode infections in the provinces of the eastern and western Cape (Fincham JE et al., 1996, Evans AC et al., 1987, Kvalsvig et al., 1991). It is reported that the children are the most vulnerable to the effects of the worm infection and revealed that the prevalence of helminthic infections as a baseline assessment were *A. lumbricoides* 29.5%, *T. trichiura* 51.9% and *S. haematobium* 22.3% among 268 school children, aged 8 to 10 in KwaZulu-Natal province (Jinabhai et al., 2001). Another study carried out among schoolchildren in northern area of the province showed that the prevalence of *T. trichiura* and *A. lumbricoides* were 57.2% and 19.4% respectively, while the prevalence of hookworms infection 83.2% which was considerably higher than in other parts of the province (Saathoff et al., 2004). There are limited data on the prevalence of intestinal parasites in the South African adult population. The stool samples from 32 randomly selected public hospital laboratories were obtained for parasitological examination in KwaZulu-Natal province. The prevalence of parasites in collected 5,733 stool samples ranged from 30% to 11.2% depending on the geographical distribution (highest infection rate in coastal region and lowest in the inland). *A. lumbricoides* (10.7%) and *T.trichiuria* (6.7%) were the most common helminth infections, followed by hookworm and *S. mansoni.* The most common protozoan parasites were *E. coli* and *E. nana.* These results might be underrepresented as those samples were collected from health facility based area; however, it illustrated relatively high prevalence of intestinal parasites in the adult population.

To date – to the best of our knowledge no epidemiological study in Laos PDR or South Africa or in fact elsewhere systematically assessed the DBD of different types of helminth infections and DM. Yet, due to the high prevalence of these diseases in Laos PDR (South-East Asia region) and South Africa (Africa region) and more generally, understanding

their mutual influence will provide important policy input for health systems planning and for caring for comorbid patients.

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Chapter 2

Research Goal, Research Hypothesis and Objectives

Goal

The overall goal of this PhD thesis is to improve the understanding of the biological and epidemiological interrelationships of dual burden of diseases in resource poor countries, i.e., chronic diseases in combination with infectious diseases. In this aspect, the focus of this work is on diabetes mellitus in combination with different neglected tropical diseases, particularly dengue viral infections and helminth infections.

Research hypothesis and research questions

We hypothesize that:

1. Diabetes mellitus increases the severity of episodes of dengue viral infections.
   Research questions
   - Is diabetes mellitus a risk factor for a severe course of dengue? Do diabetes patients have increased risk for dengue hemorrhagic fever?
   - What is current clinical and epidemiological evidence for diabetes mellitus to modulate a dengue viral infection episode?
   - What mechanisms are known that can influence the clinical course of dengue viral infection in diabetes patients?
2. Helminth infections protect from diabetes mellitus.
   Research questions
   - Do helminth infections have a protective effect against diabetes mellitus?
   - How do helminth infections improve the hyperglycemic status?
   - Do helminth species act differently on glycemic level?
3. Anthelminthic treatment increases the prevalence of diabetes in the population
   Research questions
   - Does deworming increase the glycemic level and therefore, increase diabetes prevalence of population?

Specific objectives

1. To summarize the epidemiological evidence on the association between diabetes mellitus and dengue viral infection through a systematic literature review and, meta-analyses

2. To update the current knowledge on the relationship between diabetes mellitus and helminth infections through a systematic literature review

3. To assess the association between diabetes mellitus and helminth infections among adult persons in Lao People's Democratic Republic

4. To determine the association between diabetes mellitus and chronic intestinal parasite in the children in South Africa before and after anti-helminthic treatment

Chapter 3
Methods

3.1 Overview of research methodology

Due to overlapping geographical presentation between NTDs and NCDs along with their current disease trends, we primarily expect to see the effects of NTDs occurring among patients with underlying NCDs, and hoping that it would be of a great interest to determine whether co-morbidities resulting a significant clinical presentation. To find out any potential epidemiological association between NTDs and DM, we firstly conducted a systematic literature review between dengue, one of the most emerging NTDs in recent years in the tropical countries, from published articles. Secondly, we considered to conduct a systematic review between helminthic infection which contributes a major morbidity among NTDs and DM. Then, we have come up with field studies (a cross-sectional study in Laos PDR and a longitudinal study with anthelminthic drugs in Port Elizabeth, South Africa) in order to reveal the epidemiological association between DM and helminthic infections.

3.2 Research methodology of systematic review and meta-analysis of DBD

To have a better understanding and to identify the epidemiological gap of DBD between dengue and DM, we conducted a systematic literature review using MEDLINE database to access any relevant publication describing an association between dengue and DM as a part of PhD thesis. We included articles in all languages; articles which reported on epidemiology, clinical signs, and laboratory parameters for dengue-infected patients, or on severity assessment. There was no restriction in publication dates, place of study, study design or age of research participants. As a validity assessment, the PRISMA criteria were used (PRISMA, 2014). We extracted the year during which dengue cases were diagnosed (year during which the study was conducted), year of the publication, country of the study, study design, study definitions of dengue infection and DM, and confounder adjustments. The data on the sample size of enrolled persons and number of cases and controls and on the estimates (unadjusted and adjusted models) of the association (and their 95% confidence intervals) between DM and severe dengue were also extracted. The transition of the patients data from clinical or hospital records to this review was based on published non-individual and non-identifying data. Data were extracted from the published papers independently by two reviewers and disagreements were resolved by discussion. We used random-effects models

for meta-analyses of the association between DM and a severe clinical presentation of dengue (Lau et al., 1997). They take into consideration the variation between the true effects estimated by included studies unlike fixed effect models which assume a common true effect across studies. We used odds ratios as measure of association across all studies. We used the estimates reported by the authors as "primary model" and the I^2 metric and Tau^2 to describe the between study heterogeneity and variance respectively. We conducted sensitivity analyses by using a fixed effect model and excluding a study, which was based on non-matched random controls and that only reported unadjusted estimates (Karunakaran et al., 2014). We performed analyses with Stata version 13 (Stata Corporation, Texas) and considered $p<0.05$ as statistically significant.

We used the same methodology to conduct a systematic review and explore an association between helminth infections and DM; however, retrieved data from the published articles were not good enough to perform the meta-analysis.

3.3 Research methodology of field studies in Laos PDR and South Africa

3.3.1 Study design and settings

We conducted a cross-sectional study (LADUBU study) was conducted among 1,600 adults aged in both rural and urban area of different 4 provinces. 2 provinces are located in middle part of the Laos PDR; Vientiane capital and nearby province of Luang Prabang (LP city and Namback district). The other 2 provinces are located in Southern part of the country; province of Saravanh (Saravan city and Saravanh district), and Province of Champasack (Pakse city and Kong district). In order to investigate the co-morbid association of DM and helminth infections, we surveyed the prevalence of different helminth infections and DM in rural and urban population within provinces and between provinces and the data collection was done in 2016.

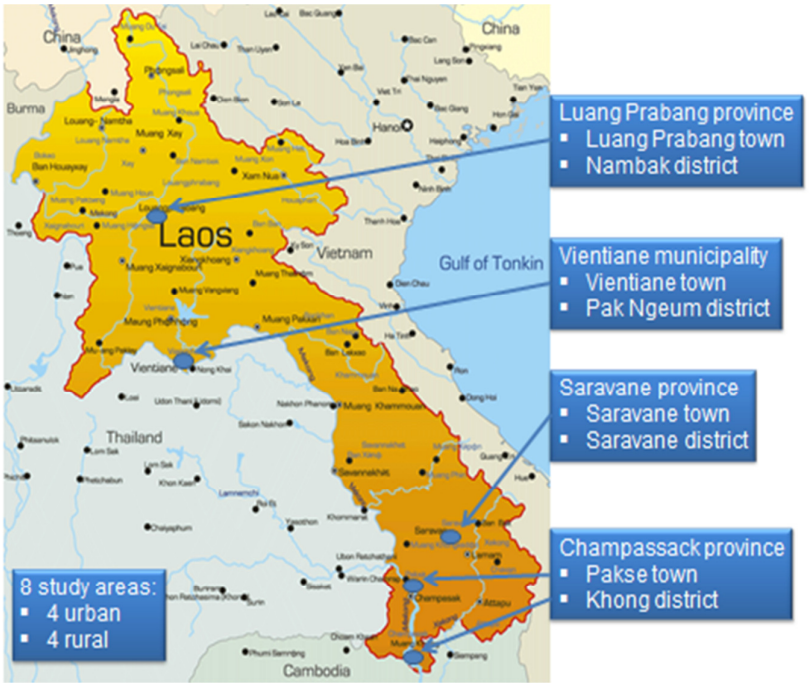

Figure 3.1: Study area of LADUBU project in Lao PDR

We conducted a longitudinal study, integrated into the ongoing DASH study with the add-on of an HbA1c protocol among 900 primary schoolchildren from 8 schools located in poor neighborhood of Port Elizabeth, South Africa. We explored the prevalence of different parasitic infections at baseline and cross-sectionally and compared blood HbA1c levels between the groups affected by helminth and other parasitic infections. The subjects affected by helminth infections were offered oral anti-helminthic drugs (Albendazole 400 mg single dose for STH infections). Mass or individual anthelminthic treatment regime was applied depending on the infectious status of the schools according to WHO recommendation. We again measured helminth infection status and HbA1c blood concentrations 6 months after anthelminthic treatment. This study was conducted in 2015-2016.

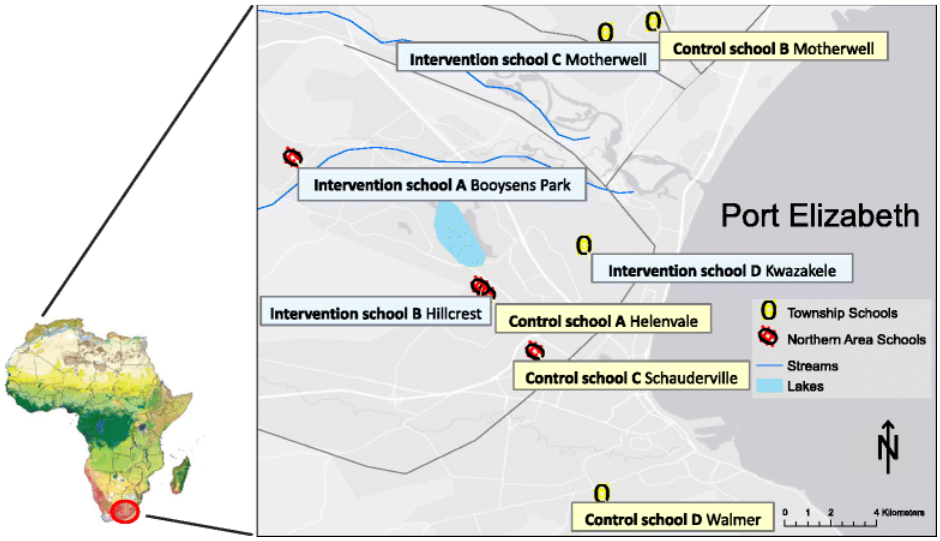

Figure 3.1: Study area and location of study schools (Yap et al., 2015)

Figure 3.2 Primary schoolchildren in one of study schools in Port Elizabeth, South Africa

3.3.2 Assessment methods of the study

Questionnaires

In both studies, the standardized questionnaires were used to capture the socio-economic status of the family, housing or living conditions and life styles by trained local volunteers who can speak the local dialect.

Clinical examination

The experienced medical professionals performed the general health assessment of participants and gathered the information about recent and current medical problems. Then, the body temperature, weight, height, blood pressure, skin fold assessment (in South African study), the assessment of anemia by using HemoCue HB 301 system to measure the haemoglobin concentration were systematically measured.

Figure 3.3 Clinical examination of in Vientiane, Laos PDR (Ladubu study)

Figure 3.4 Research team of Ladubu study

Assessment of diabetes mellitus

In both studies, we assessed DM status by evaluating HbA1c values which identify the three-month average plasma glucose concentration before the measurement and we used point-of-care instrument and the Afinion (Alere Technologies) test, which is based on

boronate affinity separation and the use of fluorescence quenching. This method met the accepted performance criteria for HbA1c as defined by the U.S. National Glycohemoglobin Standardization Program (NGSP) and no interference from other hemoglobin traits results (Westra E and RJ., 2014). All test cartridges for the Afinion tests were from the same lot number, given that recent evidence suggest a dependence of test results on the lot number (Dupuy AM et al., 2014). Only a small amount of blood (approximately 1.5µL) was withdrawn from the finger prick and the results were available in 3 minutes. The advantages of this measurement are no requirement of fasting, greater pre-analytical stability and less day-to-day perturbations during stress and illness.

Figure 3.5: Diabetes assessment with Alere techonology in South Africa project

Assessment of parasites in the collected stool samples

For the purpose of helminth assessment in LADUBU study, two stool samples from each participant were collected and fixed with 10% alcohol. The collected samples were sent to the microbiology lab in Kokean University, Thailand. Two thick smears were extracted from each stool sample with Kato-Katz method (Katz et al., 1972) and investigated the smear under a light microscopic to look for any helminth infections.

In South African study, to identify the specific STH and other parasites to increase the sensitivity of the diagnostic methods to an acceptable level, each stool sample was examined with specific diagnostic techniques, namely the Kato-Katz technique (Becker et al., 2013, Glinz et al., 2010), on the collected day. Intensity of infection were assessed by counting the eggs per Kato-Katz stool smear and multiplied by 24. A crypto-Giardia Duo-Strip Rapid

Diagnostic test (RDT) were used to detect Cryptosporidium species and *Giardia intestinalis.* To assess *S. haematobium* infection, single urine sample from each child were collected and analyzed visually for macro-haematuria and tested with reagent strips to detect the blood in urine. In addition, to confirm the presence of *S.mansoni* infections, the point-of-care circulating cathodic antigen (POC-CCA) test was performed. Urine infiltration and kato-katz samples were randomly re-checked for quality assurance of the diagnostic accuracy of results (Knopp et al., 2008).

3.4 Research collaboration and ethical statement

The Laos project was a cross unit internal collaboration between Swiss TPH (Chronic Diseases Epidemiological Unit and Ecosystem Health Sciences Unit and joint research collaboration between Swiss Tropical and Public Health Institute (Swiss TPH) and the Francophone Institute for Tropical Medicine (IFMT). The ethical approval of study protocol was obtained from the Lao Ethical Review Committee with agreement of provincial and district health authorities to conduct the study.

The South African project was also the same research internal collaboration within Swiss TPH but an external collaboration between Swiss TPH and, University of Basel (department of sport science) and Nelson Mandela Metropolitan University (Health Science Faculty, the department of Human Movement Science) in Port Elizabeth, South Africa. Our study has been integrated into an ongoing study entitled “Impact of disease burden and setting-specific interventions on schoolchildren` cardio-respiratory physical fitness and psychosocial health (DASH study)”. The proposed add-on HbA1c test results can additionally increase the scientific output of the DASH project. The DASH study had obtained ethical approval from Ethikkommission Nordwest und Zentralschweiz (EKNZ) according to Swiss TPH regulations, as well as from ethics committees in South Africa (NMMU health Sciences Faculty Research committee, NMMU Human Ethics Committee, Eastern Cape Department of Education (as the study was conducted at the schools) and the Eastern Cape Department of Health). The written informed consent from the research participants (children parents or guardians in South African study) was obtained prior the data collection, to capture socio-economic and demographic information using questionnaires and also biological samples.

All participants infected with helminth infections were provided the anthelminthic treatment with Alendazole 400 mg according to WHO guideline. The participants who have higher HbA1c levels than normal cut-off values were also referred to local hospital or clinic

where their DM status could be confirmed and they could receive the treatment when necessary.

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Chapter 4

Is diabetes a risk factor for a severe clinical presentation of dengue?

Review and meta-analysis

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4.1 Abstract

Background

The mean age of acute dengue has undergone a shift towards older ages. This fact points towards the relevance of assessing the influence of age-related comorbidities, such as diabetes on the clinical presentation of dengue episodes. Identification of factors associated with a severe presentation is of high relevance, because timely treatment is the most important intervention to avert complications and death. This review summarizes and evaluates the published evidence on the association between diabetes and the risk of a severe clinical presentation of dengue.

Methodology/Findings

A systematic literature review was conducted using the MEDLINE database to access any relevant association between dengue and diabetes. Five case-control studies (4 hospital-based, 1 population-based) compared the prevalence of diabetes (self-reported or abstracted from medical records) of persons with dengue (acute or past; controls) and patients with severe clinical manifestations. All except one study were conducted before 2009 and all studies collected information towards WHO 1997 classification system. The reported odds ratios were formally summarized by random-effects meta-analyses. A diagnosis of diabetes was associated with an increased risk for a severe clinical presentation of dengue (OR 1.75; 95% CI: 1.08-2.84, p =0.022).

Conclusions/Significance

Large prospective studies that systematically and objectively obtain relevant signs and symptoms of dengue fever episodes as well as of hyperglycemia in the past, and at the time of dengue diagnosis, are needed to properly address the effect of diabetes on the clinical presentation of an acute dengue fever episode. The currently available epidemiological evidence is very limited and only suggestive. The increasing global prevalence of both, dengue and diabetes, justify further studies. At this point, confirmation of dengue infection as early as possible in diabetes patients with fever if living in dengue endemic regions seems justified. The presence of this co-morbidity may warrant closer observation for glycemic control and adapted fluid management to diminish the risk for a severe clinical presentation of dengue.

4.2 Author Summary

Both, dengue and diabetes have reached epidemic dimensions and pose a joint threat to a large proportion of populations in low- and middle-income countries. Dengue is no longer a disease primarily affecting children. Therefore the influence of non-communicable diseases such as diabetes, which are increasingly prevalent in adults, on the clinical presentation of a dengue episode becomes a public health priority. We conducted a systematic literature review to assess the available evidence on the effect of diabetes mellitus (DM) on the clinical presentation of dengue. The meta-analysis of published evidence combined with supporting biological evidence point to an increased risk for potentially life threatening symptoms of dengue among patients with diabetes. The current evidence is limited by statistical power and other study limitations and does not allow conclusions about a causal effect of diabetes. Yet, based on the currently available evidence, diabetes patients with fever and living in a dengue endemic region should seek confirmation of dengue infection as early as possible. Diabetes should be considered in the triage of patients for close observation and early intervention, which are challenges, particularly during dengue outbreaks. Timeliness of intervention is the most important factor averting serious complications and death in patients with acute dengue.

4.3 Introduction

With low- and middle-income countries (LMIC) experiencing a growing chronic non-communicable disease (NCD) burden and a continuously high communicable disease (CD) incidence rate, understanding the co-morbidity between the two disease groups is necessary to properly assess, monitor, evaluate, and control their prevalence (1, 2). Cardiovascular diseases and their risk factors including diabetes mellitus (DM) are major contributors to the growing NCD burden (3). WHO projects that DM will be the 7th leading cause of death in 2030. Today there are 347 million people worldwide who have DM (4), around 90% of them type 2 DM (5). More than 80% of DM deaths occur in LMIC(5). In high income countries DM has long been known for its association with increased susceptibility to infections such as tuberculosis (6). Although these associations have been attributed in part to DM associated alterations in innate immunity, related evidence is inconsistent and underlying mechanisms remain poorly understood. They are likely to vary by type of infection (7). Yet, only few studies investigated the complex associations of diabetes with neglected tropical diseases (NTDs).

Dengue, one of 17 diseases assigned NTD status by WHO, is next to malaria the most important arthropo-borne (ARBO) tropical infection caused by the dengue virus. It is transmitted by several mosquito species within the genus *Aedes*, principally *A. aegypti* (8). The number of Dengue virus infections has increased 30fold over the last decades. Today it is a major public health problem in tropical and subtropical regions (9). The absence of adequate public health awareness, surveillance and control, population growth, globalization and urbanization contributed to this increase. An estimated 2.5 billion people are at risk of infection in over 100 endemic countries(10). WHO estimates that currently between 50 and 100 million dengue infections occur annually. An estimated 500'000 dengue patients with potentially life threatening symptoms require hospitalization each year and about 2.5% of those affected die (11). Dengue ranges from asymptomatic or self-limiting non-severe dengue (with or without warning symptoms) to severe dengue, characterized variously by severe plasma leakage, severe bleeding or severe organ involvement (12). The studies reviewed in this paper were all except one conducted before 2009 and therefore routine clinical data was collected according to the WHO 1997 guidelines. These guidelines group symptomatic dengue virus infections into three clinical categories: undifferentiated fever, dengue fever (DF), and dengue hemorrhagic fever (DHF). DHF is further subclassified into four severity grades, with grades III and IV being defined as dengue shock syndrome (DSS) (13). The clinical presentation of a dengue infection is difficult to predict, although the presence of warning signs occurring within 3 to 7 days after the first symptoms warrant strict observation

and medical intervention. Intervention, including intravenous rehydration as the therapy of choice, can reduce case fatality in severe dengue to less than 1 % (14). Dengue has long been viewed as a pediatric disease, but the average age of dengue cases has been rising and there is a suggestion for adults to be at increased risk for dying from dengue. The increase of tourism in tropical regions also contributed to the increase in adult dengue cases (15) .

There are five dengue virus (DENV) serotypes (DENV-1, DENV-2, DENV-3, DENV-4 and DENV-5), however, serotype 5 seems not to have a sustained transmission cycle in humans (16). Infection confers immunity to the infecting serotype which is life-long, but not to the remaining three. Subsequent infections with a different dengue virus serotype increase the risk of severe complications (17). Other than that, factors increasing the risk of severe clinical manifestations remain poorly characterized (18-20). Present evidence suggests that beyond viral factors age, gender, social status, genetic background, sickle cell anemia, uremia, bronchial asthma, allergies, hypertension, chronic renal failure and also DM might adversely influence the clinical presentation of an infection (18, 19, 21-24).

In acknowledging the shift of dengue to older ages and the steep increase in the prevalence of DM, the objective of this review is to access the current clinical and epidemiological evidence for this NCD to contribute to a higher risk of a severe clinical presentation in dengue fever patients.

4.4 Methods

Search strategy

We conducted a systematic literature review using MEDLINE database to access any relevant publication describing an association between dengue and diabetes up to February 28 2014. The search terms used were “("dengue"[MeSH Terms] OR "dengue"[All Fields]) AND ("diabetes mellitus"[MeSH Terms] OR ("diabetes"[All Fields] AND "mellitus"[All Fields]) OR "diabetes mellitus"[All Fields] OR "diabetes"[All Fields]”.

Inclusion criteria

We included articles in all languages; articles which reported on epidemiology, clinical signs, and laboratory parameters for dengue-infected patients, or on severity assessment. There was no restriction in publication dates, place of study, study design or age of research participants. As a validity assessment, the PRISMA criteria were used (25, 26).

For meta-analyses, we included studies that compared the prevalence of DM between persons affected by different dengue stages (case-control studies), reporting estimates of association and their 95% confidence intervals, or enough information to derive this.

Data extraction

We extracted the year during which dengue cases were diagnosed (year during which the study was conducted), year of the publication, country of the study, study design, study definitions of dengue infection and diabetes, and confounder adjustments. We extracted data on the sample size of enrolled persons and number of cases and controls and on the estimates (unadjusted and adjusted models) of the association (and their 95% confidence intervals) between diabetes and severe dengue. The transition of the patients data from clinical or hospital records to this review was based on published non-individual and non-identifying data. Data were extracted from the published papers independently by two reviewers and disagreements were resolved by discussion.

Case Definitions

Dengue

The studies included in this review mostly included dengue diagnosed before 2009 and applied the WHO 1997 dengue classification criteria (13). A 2009 classification was proposed by the WHO/TDR group and in 2011 the WHO/SEARO group also suggested modifications (14). We list the classification system applied in the respective studies in Table 1, which summarizes the available evidence. Control status and case status are predominantly defined as DF and as DHF or DSS respectively. Cases of DHF/DSS in the studies reviewed were hospitalized cases or deaths confirmed serologically, clinically with hemorrhagic manifestations or radiographically with a certain extent of plasma leakage. According to WHO 1997 classification, DHF is clinically subdivided into grades I-IV and all 4 grades could have some degree of at least subclinical plasma leakage. Grade I is the presence of fever with positive tourniquet test, grade II presents with spontaneous bleeding into the skin and elsewhere, grade III shows clinical signs of shock or circulatory failure, and grade IV presents with severe shock with undetectable blood pressure and pulse. Grade III and IV have also been labeled as “dengue shock syndrome” (DSS). The WHO 2009 classification differs from the 1997 classification scheme in that dengue cases are classified by the levels of severity with or without warning signs. Several clinical features specifically listed in the WHO 2009 classification were not directly obtained in the reviewed studies, e.g. clinical features such as lethargy, restlessness, and severe organ impairment or failure. We refer to DHF/DSS as “severe clinical presentation of dengue”.

In the reviewed publications, laboratory diagnosis methods to confirm dengue virus infection as the cause of disease varied across studies and involved detection of virus, viral nucleic acid, antigens or antibodies, or a combination of these techniques (12). The detection

of virus, nucleic acid or antigen in the blood is confirmatory for an acute dengue infection. Antibody response to infection differs by the host immune status. In the setting of a primary infection, IgM are the first antibodies to appear, but it takes 10 days for them to be detectable in 99% of patients. During a secondary dengue infection, anti-dengue IgG are detectable at high levels already in the acute phase, whereas IgM levels remain significantly lower than in primary dengue infection. A four-fold or greater increase in IgG antibody levels in paired sera (sample collected during the acute stage of illness and sample collected in the convalescent stage) indicates acute dengue (12). Identification of virus/viral RNA/viral antigen and antibody response are ideally combined for the confirmation of acute dengue. Yet, as specimen collection and processing is easier for serology, the laboratory methods applied in the studies mostly conducted in LMIC varied broadly. We list the laboratory confirmation methods applied in the respective studies in Table 1, together with the available evidence.

Diabetes Mellitus

In studies included in this review, the diagnosis of DM was mostly ascertained by self-reported diagnosis in the context of in-person interviews or DM diagnosis listed in clinical records. The DM diagnosis was further verified by requesting the prescription and/or medication package during the in-person interview in the only population-based case-control study (27). An oral glucose tolerance test was performed in dengue patients in the case series published by Hasanat study (28).

Meta-analysis

We used random-effects models for meta-analyses of the association between DM and a severe clinical presentation of dengue (29). They take into consideration the variation between the true effects estimated by included studies unlike fixed effect models which assume a common true effect across studies. We used odds ratios as measure of association across all studies. We used the estimates reported by the authors as “primary model” and the I^2 metric and Tau^2 to describe the between study heterogeneity and variance respectively.

We conducted sensitivity analyses by using a fixed effect model and excluding a study, which was based on non-matched random controls and that only reported unadjusted estimates (30). We performed analyses with Stata version 12 (Stata Corporation, Texas) and considered $p<0.05$ as statistically significant.

4.5 Results

Our literature search resulted in 32 hits (Fig. 1). After excluding duplicates and non-relevant articles based on a full text analysis (single case reports, outbreak investigation reports, no specific factors studied, narrative overview of dengue infections in a specific

country or globally), ten articles were retained. Among them, five studies were case-control studies (30-33) with one study being population-based (27). The case-control studies compared the prevalence of DM in persons with acute or past non severe dengue (controls) to that in persons with an acute severe clinical presentation of dengue. Five additional articles (case series) characterized DM in mostly dengue patients with severe clinical manifestations, including fatal cases (28, 34-37). These studies lack a comparison group with acute or past non severe of dengue, and are therefore not included in the meta-analysis.

Epidemiological evidence from case-control studies on the association between DM and a severe clinical presentation of dengue

Epidemiological studies included in the meta-analysis compared the prevalence of DM and other co-morbidities between patients suffering from acute dengue with a severe presentation and controls with acute or past dengue without severe clinical manifestations (Table 1). In the absence of controls without evidence for a dengue infection history, studies thereby compared the prevalence of DM in dengue patients with different degrees for severity in clinical presentation, rather than the risk of being infected with dengue virus.

The only population-based case-control study (27) identified was conducted in two Brazilian cities and included both, children and adults. DHF cases were ascertained through the national surveillance system. The surveillance records were reviewed by two physicians. DHF was defined according to criteria used by Brazilian Healthy System, which were very close to WHO 1997 criteria. Controls were selected from the same neighborhood as cases. In addition, they were tested positive for anti-dengue IgG and matched to cases by age, sex and a report of a past dengue-like fever in the same year as the DHF diagnosis of cases. Additional information from both, cases and controls was obtained through in-person interviews. DM was based on a self-reported physician-diagnosis. Interviewers also asked for medication intake and verified it by seeing the prescription of packaging. DM was statistically significantly associated with DHF independent of age, sex skin color, income and educational level (aOR 2.75, 95% CI 1.12-6.73). The association of self-reported diabetes with DHF was stronger in diabetic patients being treated, especially if treated with insulin or more than 1 drug (aOR 3.36; 95% CI 0.72-15.61). In addition, white ethnicity (aOR 4.70, 95% CI 2.17-10.2), high income (aOR 6.84, 95% CI 4.09-11.43), high educational level (aOR 4.67, 95% CI 4.09-11.43) and a self-report of allergy treated with steroids (aOR 2.94, 95% CI 1.01-8.54) were also associated with a more severe clinical presentation of dengue.

A hospital-based study including persons aged 15 to 65 in Pakistan (31) compared hospitalized acute DHF patients (cases) to dengue IgG positive patients, but hospitalized for

unrelated conditions (controls). The study was conducted in two major tertiary care hospitals. A DHF case was defined as diagnosis by an experienced clinician applying WHO criteria, although the version applied was not specified. The categorization into persons with and without DHF points to the use of WHO 1997 criteria. Information was obtained through structured record review and in-person interview. Age- and sex-matched DHF cases were slightly more likely to report a DM diagnosis than control patients. In both groups, the reported prevalence of DM was exceptionally high, 41.8% in controls and 43.2% in cases. The association of DM with DHF adjusted for age, sex and duration of illness was statistically non-significant (aOR 1.26, 95% CI 0.78-2.03, p= 0.34).

In Singapore, a hospital-based study was conducted in the nation's largest clinic. It included all admitted patients with acute dengue without age restriction (32). Information on case and control status as well as comorbidities was exclusively derived from abstracting medical charts. Cases were defined as DHF patients, and controls were DF patients. Probable dengue patients had a positive acute dengue serology. Confirmed dengue patients had positive dengue polymerase chain reaction assays. Clinical diagnosis for DHF was based on WHO 1997 criteria. The data was analyzed separately for the two epidemic periods of 2006 (predominantly DENV-1) and 2007/2008 (predominantly DENV-2; larger sample size). No association between DM status and DHF was found in the 2006 dengue outbreak. DM was independently associated with DHF in the 2007/8 epidemic (aOR 1.78, 95% CI 1.06-2.97). The association was stronger if the diabetic patients additionally had hypertension (aOR 2.16, 95% CI 1.18-3.96) or asthma (aOR 4.38, 95% CI 0.80-23.85). Mean hospitalization days were longer for DM (4.99±3.34days) as compared to non-DM patients (4.04±1.62 days, p =0.001). Additional factors associated with DHF were Chinese ethnicity (compared to Malay or Indian ethnicity) (aOR 1.90, 95% CI 1.01-3.56 in 2006 epidemic periods and aOR 1.67, 95% CI 1.24-2.24 in 2007/2008 epidemic periods) as well as middle age in 2007/2008 (aOR 1.41, 95% CI 1.09-1.81 in 30-39 years and aOR 1.34, 95%CI 1.09-1.81 in 40-49 years of age group).

In 2002 in Taiwan, all patients with confirmed acute DF treated at the Kaoshiung Medical University Hospital during a large outbreak occurring in the southern Taiwan were categorized into groups of DF and DHF/DSS by strictly adhering to clinical WHO 1997 criteria and laboratory confirmation under the auspices of the Taiwanese Centre of Disease Control (33). Clinical information such as signs and symptoms and the results of blood investigation were abstracted from medical records. Cases were mostly adults, only 4.5% were below age 15 years. The prevalence of DM was 16.8% in DHF/DSS cases compared to

7.6% in DF patients (controls). The adjusted OR reported was 1.86 (95%CI 1.04-3.37), albeit covariates were not reported. In addition to the independent association of DHF/DSS with DM, statistically significant associations with hypertension and renal insufficiency, uremia, past history of dengue infection as well as male gender and older age were also found.

A hospital-based study in Southern India (30) obtained information on patients admitted to the largest multi-specialty hospital in South Kerala for acute dengue between 2005 and 2008. The case group consisted of 10 in-hospital deaths of patients admitted with a clinical diagnosis of probable dengue, which was confirmed by either RT-PCR or IgM antibody tests, and review of clinical symptoms through medical record review. Forty non-matched controls were randomly selected among patients with a confirmed acute dengue, but recovering from the illness. The classification of dengue among the controls was not specified. Information on co-morbidities and other factors was abstracted from medical records. The prevalence of DM in controls was 2.5% compared to 40% in cases. DM was a strong predictor of mortality in the bivariate analysis (OR 26.0, 95% CI 2.47-273.67, p =0.004). In the same study, hypertension was also a strong predictor of mortality (OR 44.3, 95% CI 6.2-315.5, p= 0.000). Mortality was much higher in patients over 40 years (OR 9.3, 95% CI 1.9-44.4, p= 0.002). No adjusted odds ratios, which would facilitate the interpretation of independency in the reported associations, were reported.

Meta-analysis of epidemiological evidence from case-control studies

We included the results of five above mentioned studies in a meta-analysis. One of these studies reported two separate estimates from two independent cross-sectional assessments; hence, we considered them as separate studies. The meta-analysis showed that the presence of a severe clinical presentation of dengue was positively associated with the presence of DM.

A diagnosis of DM was associated with an increased risk for severe clinical manifestations of dengue by 75% (95% CI: 1.08-2.84, p =0.022) compared to non-DM patients (Fig. 2). This OR remained robust across sensitivity analyses involving fixed effect analysis. We observed some heterogeneity across the studies, consistent with the broadly differing study settings. The small number of studies included in the meta-analysis did not provide statistical power for formal statistical assessment of heterogeneity (Fig. 2).

Clinical case series on characteristics of DHF patients

We additionally identified five case series reporting dengue-related hospitalizations and the prevalence of DM in these cases. They are listed in Table 1 as reference of the prevalence of DM in dengue patients with a severe clinical presentation. The studies have

been conducted mostly in Asian dengue endemic regions such as Malaysia (36); Bangladesh (28); Singapore (34, 35). In the absence of a control group, these studies are of limited value for better understanding of the role of DM on the clinical presentation of dengue. In several instances the case definition was restricted to just being dengue seropositive. This does not allow differentiating between DM influencing the clinical manifestations of dengue infection versus dengue infection influencing the clinical presentation of DM. Of interest, in that respect is the study by Hasanat et al (28). Hospitalized DF patients underwent oral glucose tolerance testing (OGTT) between 3 and 10 days after the start of illness. A subset of these patients agreed to a second OGTT before discharge. The authors demonstrated a high rate of glucose intolerance in the early phase of disease, which returned though to normal in 55% of the patients.

4.6 Discussion

The few published studies specifically addressing the role of DM as a risk factor for a severe clinical presentation of dengue provide suggestive evidence for an adverse effect. This result merits further investigation in the context of study designs that overcome the weaknesses of currently available publications as addressed below.

Assigning causality to the modifying effect of diabetes on the clinical presentation of dengue is premature. First, the case-control studies conducted to date and summarized above are mostly retrospective in nature. The clinical and laboratory diagnostic criteria applied in different studies vary broadly, as does the definition for the control group. Second, the WHO 1997 dengue classification used in the studies reviewed, has itself a number of limitations. For example, the development of this classification was based on disease patterns of children in Thailand, potentially limiting its generalizability to other geographical regions and to older age groups. Furthermore, clinical assessments such as the Tourniquet test do not differentiate between DF, DHF and other febrile illness (38, 39). It also fails to detect severe dengue manifestations in many patients (40). Third, information on DM was either self-reported or record-based and did not systematically allow differentiating between DM diagnosed before versus concurrent with the dengue episode. Given the high degree of under diagnosis of DM, especially in LMIC, misclassification of DM status in the studies is likely substantial. The problem of under diagnosis is not easy to overcome, as DM measured at the time of acute dengue is not necessarily reflecting the underlying DM status, but rather stress-related hyperglycemia, which disappears after recovery from dengue (28). Longitudinal studies of dengue patients are needed that allow studying both, the longitudinal course of hyperglycemia and the evolvement of clinical presentation of the dengue infection. In addition, whether better

control of DM in early stages of dengue will prevent severe forms of dengue needs to be assessed in the context of intervention studies. The basis should be dengue surveillance programs which registers all dengue episodes and which includes routine collection of blood specimens for HbA1c and other hyperglycemia parameters and of information regarding diagnosis and treatment of DM. A fifth limitation of studies conducted to date is the fact that most of them were hospital-based case-control studies with a high potential for selection bias. But the results from the only population-based case-control study also point to DM increasing the risk for a severe clinical presentation of dengue (27). Finally, study limitations also include the fact that the modifying effect of DM on different dengue serotypes could not be differentiated; that the age range of study subjects was not always reported; and that many studies included children and adolescents in whom DM is rare.The observed heterogeneity between the results of the individual studies is a limitation in the meta-analysis and reflects differences in the diagnostic and classification criteria for dengue and DM, differences in the controls selected, as well as differences in study design such as sample size, population and study setting or confounders considered in the analysis. Our systematic and broad search strategy, (not limiting to publication dates, place of study, study design or age of research participants) and the novelty of this topic are the strengths of our review and should stimulate further research into the topic, also in the light of supporting results from experimental studies.

Biological evidence exists to support the hypothesis for a high DHF: DF ratio in diabetes. One example for a line of reasoning is that the cytokine overload related to a Th1 to Th2 shift and a severe manifestation of dengue superimposed by the cytokine overload of a diabetic state may be particularly detrimental to the endothelium and for subsequent vascular leakage (41-43). The third space fluid shift such as pleural/pericardial effusion or ascites is an important clinical manifestation in dengue with severe clinical symptoms, which is a consequence of endothelial dysfunction and results in hemoconcentration, hypotension and shock (34). DM shares with a severe clinical presentation of dengue alterations in the innate immune response, a pro-inflammatory state and endothelial dysfunction. Lee and colleagues infected mononuclear cells from diabetic (n=33) and healthy individuals (n=29) with dengue virus. Cells from diabetics produced higher levels of IL-4 and of both, IL-4 and IL-10 as well as granulocyte-macrophage colony-stimulating factor (GM-CSF) on the first and third day post-infection, respectively. No differences in viral load were found (44). In a post-mortem study of DHF/DSS related deaths, the intestinal serosa of 1 DM case with ascites, but not of non-DM cases showed apoptosis of microvascular endothelial cells (42). Microvascular

endothelial cells cultured with sera from acute dengue infection patients containing high TNF-α levels exhibited activation and apoptosis, a pathophysiological alteration likely related to vascular leakage (41).

Currently available epidemiological studies on the DM/DHF, DSS association do not provide sufficient clinical details to specifically address this "cytokine overload" hypothesis. For example, the Brazilian study explicitly stated that it did not look into ascites or pleural effusion, as these were very rarely recorded (27). The other studies did not mention plasma leakage such as pleural effusion or ascites except for the Taiwan study, where 51% of 164 DHF cases showed evidence of pleural effusion in X- ray and 31% of DHF cases had ascites in ultrasound (33). Future studies thus need to specifically address the issue of plasma leakage in the overlap between severe dengue and DM. Clinical reporting of the presence of pleural effusion and ascites as signs of severe plasma leakage is essential, as hemoconcentration and shock symptoms in the absence of plasma leakage can also arise from poorly controlled DM. The direct hypovolemic effect of hyperglycemia and the associated elevated hematocrit may even lead to misclassification of diabetics as DHF in the absence of objective evidence of plasma leakage. While recognition of hemoconcentration irrespecitive of its reason is of primary clinical importance for the provision of appropriate fluid therapy, currently available studies on the DM and DHF/DSS association do not provide sufficient clinical details to differentiate between effects of DM vs. DHF/DSS.

The potential role of altered innate immunity in mediating the association between DM and a severe clinical presentation of dengue obtains indirect support by observations that the latter may also have a higher prevalence of asthma and allergies. Patients with asthma and allergy also exhibit altered Th1 and Th2 responses (27, 31). But in DM, factors beyond the altered cytokine profile may additionally put people at risk. Diabetic patients express high rates of hypertension and impaired renal function which are both themselves associated with endothelial dysfunction (45, 46). Hypertension was also observed to be more common in patients with dengue (27, 30-32, 35), but often exhibited no independent effect in regression models adjusting for diabetes (31, 32). Pang and colleagues (2012) reported a higher risk for a severe clinical presentation of dengue in hypertensive when compared to non-hypertensive diabetic patients (32).

4.7 Conclusion

Understanding factors increasing the likelihood of dengue patients with severe clinical symptoms would help the physician to decide in a timely fashion on the need for close observation, adequate treatment, or hospitalization. The available evidence points to DM as a

potentially important co-factor. Additional prospective studies among DM and non-DM patients are needed to assess the impact of pre-existing DM as well as of hyperglycemia at the time of dengue diagnosis on the risk of a severe clinical presentation of dengue and of related deaths. Even in the absence of causal inference, it seems justified that fever episodes in patients with DM and living in a dengue endemic region are confirmed for dengue as soon as possible and that they remain under close surveillance if an acute dengue infection is confirmed. Future studies need to also address whether better control of glycemia level in dengue patients with DM can improve the outcome of the patient and decrease the risk of a severe clinical presentation. As the fluid management in diabetic patients with a severe clinical presentation of dengue poses a particular challenge, this issue should be taken into consideration by these studies. Diabetic patients in dengue endemic regions should consistently receive recommendations to protect against dengue infection by taking preventive measures against indoor mosquito breeding and against mosquito bites.

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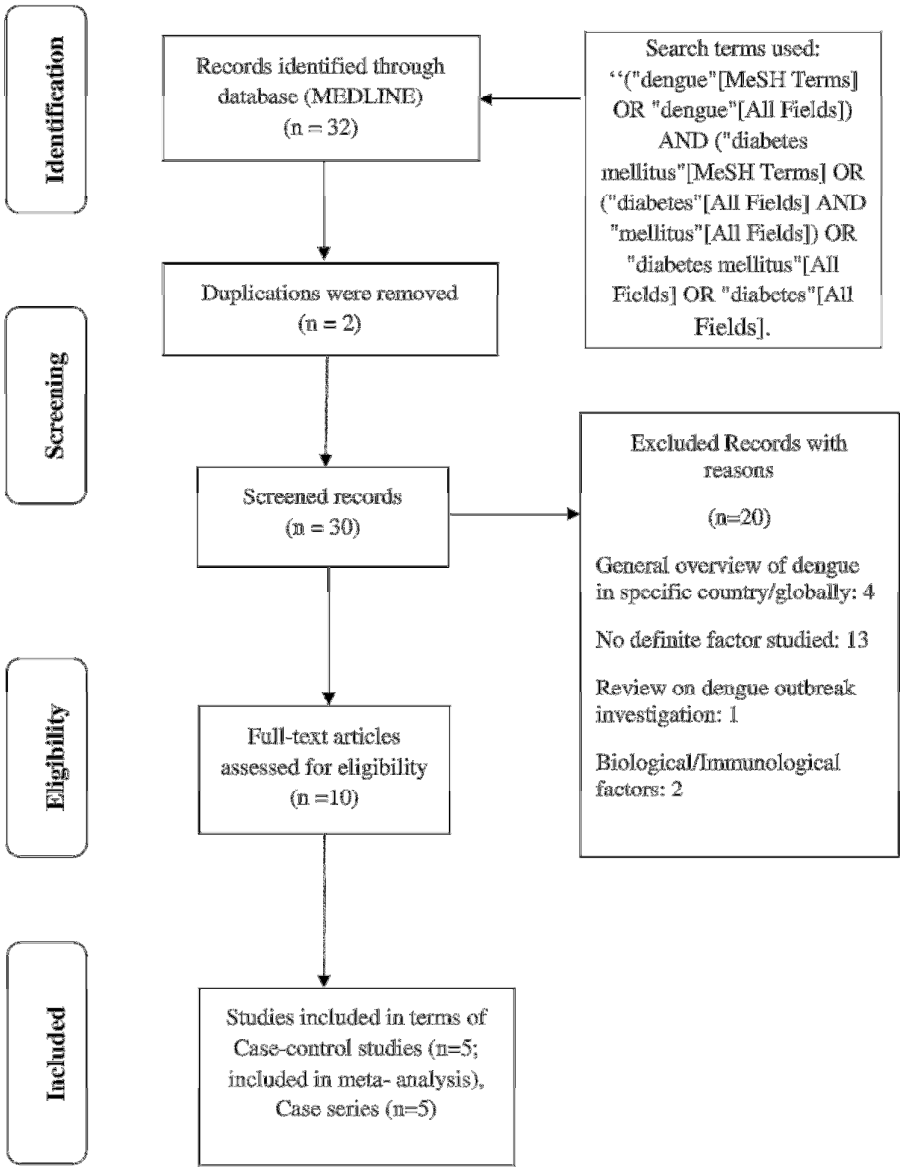

Fig. 1: PRISMA Flow diagram of diabetes and dengue

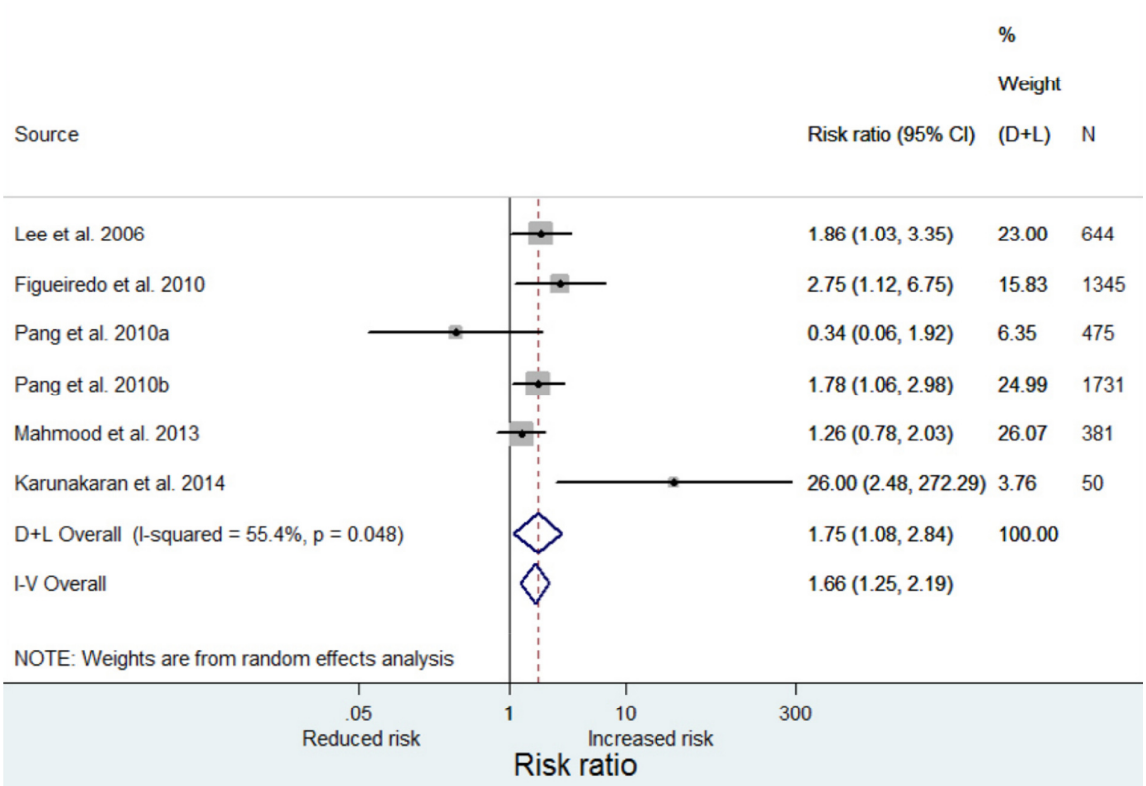

Fig. 2: Meta-analysis of case-control studies on the association between diabetes mellitus and a severe clinical presentation of dengue

Table 1. Summary of case-control studies and case series

First author and publication date	Country & Year of study	Study design	Characteristics of Study population	Results(Odds ratios (OR) and 95% confidence intervals)
Epidemiological case-control studies				
Figueiredo et al.(2010)	Brazil, 2002-2003 (Salvador), 2003-2005 (Fortaleza)	Population-based case-control study	**Cases**:170 acute DHF -registered in the national surveillance systems -residents of 2 cities -no age restriction -diagnostic criteria: surveillance record review by 2 physicians; Brazilian Health Service (very similar to WHO 1997) criteria; fever & positive serology for anti-dengue IgM and/or viral isolation and characterization; at least two signs or symptoms of dengue fever (headache or retroorbital pain, myalgia, arthralgia, prostration, exanthema); all of the following signs: hemorrhagic manifestations, hemoconcentration with an increased haematocrit level; thrombocytopenia; no consideration of ascites or pleural effusion (rarely recorded) **Controls**: 1175 -neighborhood controls -sero-positive (anti-dengue IgG) -self-report of dengue like illness in same year as matched case -no history of DHF -matched for age and sex (within 5 years) **Information on dengue history, confounders, effect modifiers & comorbidities**: in-person interviews with cases and controls **DM**: self-report of physician diagnosis and verification of prescription /packaging of medication	Predominantly DENV-3,Association DM – acute DHF vs. asymptomatic (IgG) (+)ve controls Adjusted OR (age, sex,income, neighborhood, skin colour, education) DM yes vs. no.: aOR 2.75 (1.12-6.73) DM according to the number of medications: no medication vs. no DM :aOR 1.83 (0.18-18.67) 1 medicine vs. no DM: aOR 2.72 (0.86-8.60) Insulin/ >1 medicine vs. no DM:aOR 3.36 (0.72-15.61) DM prevalence: controls:2.6%,cases:5.3%

Mahmood et al (2013)	Pakistan (2011)	Hospital-based case-control study	**Cases**: 132 acute DHF -admitted to two major tertiary care hospitals of Lahore -age 15-65 -diagnostic criteria: diagnosed as DHF by a trained clinician; WHO criteria (version not specified) **Controls**: 249 patients without acute dengue -random sample of patients from same health facilities admitted for reasons other than dengue; -positive for anti-dengue IgG; -matched for age and sex (within 5 years); -Information on dengue history, confounders, effect modifiers & co-moribities: in-person interviews with cases and controls; checklist for clinical record review **DM**: self-report or clinical record	Association DM – acute DHF vs. asymptomatic IgG positive controls: Adjusted OR (sex, age, duration of illness) DM yes vs. no: aOR 1.26 (0.78-2.03), p=0.34 DM according to its duration: 5-10 vs. <5 yrs aOR 2.76(0.77-9.84),p=0.11 >10 vs. <5 yrs:aOR 1.86 (0.55-6.26),p=0.31 DM prevalence: controls: 42% ,cases:43%
Pang et al (2010)	Singapore (2006-2008)	Hospital-based case-control study	2006 epidemic: Cases:149 acute DHF, Controls:326 acute DF 2007/2008 epidemic: Cases:590 acute DHF, Controls:1141 acute DF -admitted to the largest hospital of Singapore for dengue -adult -diagnostic criteria: **probable DF**: positive acute dengue serology (Dengue Duo IgM & IgG Rapid Strip Test) and clinical criteria of DF by WHO1997; **confirmed DF**: positive dengue PCR and clinical criteria of DF by WHO 1997 -**DHF**: presence of all four criteria of fever, hemorrhagic manifestiations, thrombocytopenia, plasma leakage **Information on dengue history, confounders, effect modifiers & comoribities:** clinical record review **DM**: clinical records	2006 epidemic: predominantly DENV-1 27.6% of patients PCR (+)ve 72.4% of patients sero-positive & PCR(-)ve Association DM- acute DHF vs. acuteDF: Adjusted OR (age, ethnicity) DM yes vs. no: aOR 0.34 (0.06-1.89) DM prevalence: controls: 2.2% ,cases: 1.3% 2007/8 epidemic: predominantly DENV-2 32.6% of patients PCR positive 67.4% of patients sero-positive & PCR (-)ve Association DM- acute DHF vs. acute DF: aOR (age, ethnicity, gender, hypertension) DM yes vs. no: aOR 1.78 (1.06-2.97) DM with hypertension: aOR 2.16 (1.18-3.96) DM with hyperlipidemia:aOR 1.62 (0.90-2.92) DM with asthma: aOR 4.38 (0.80-23.85) DM prevalence: controls:3.5% ,cases:6.4%

Lee et al. (2006)	Taiwan (2002)	Hospital-based case-control study	**Cases**:232 acute DHF (12 DSS) **Controls**:412 acute DF -all confirmed acute DF treated at Kaohshiung Medical University Hospital in 2002 -no age restriction -diagnostic criteria for confirmed acute **DF**: WHO 1997 criteria, meeting any of: positive dengue virus by PCR; 4-fold increase of dengue virus-specific IgM or IgG in paired serum samples; positive for dengue virus-specific IgM or IgG in a single serum sample; additional diagnostic criteria for confirmed acute **DHF**: Thrombocytopenia; Evidence for hemorrhage and plasma leakage; additional diagnostic criteria DSS: hypotension, narrow pulse pressure, clinical signs of shock **Information on dengue history, confounders, effect modifiers & comoribities:** clinical record review **DM**: clinical records	Association DM- acute DHF/DSS vs. acute DF: Adjusted OR(factors not reported) DM yes vs. no: aOR 1.86 (1.04-3.37) DM prevalence: cases :16.8% ,controls:7.6% Plasma leakage prevalence: Pleural effusion: 51% of DHF cases Ascites: 31% of DHF cases
Karunakaran et al (2014)	India (2005-2008)	Hospital-based case-control study	**Cases**: 10 acute dengue patients, fatal **Controls**: 40 acute dengue patients, non-fatal -confirmed dengue patients admitted to in South Kerala hospital between 2005-2008 diagnostic criteria: -confirmation by PCR or IgM antibody **Information on dengue history, symptoms, confounders, effect modifiers & comorbities:** semi-structured interview ;assessment of warning signs according to WHO 2009 case definition **DM** mellitus: self-report	Association DM - mortality among confirmed acute dengue patients DM yes vs. No: Crude OR 26.0 (2.5-273.7) DM prevalence: controls:2.5%, cases:40%

Case series				
Lye et al. (2009)	Singapore (2004)	Hospital-based	**Cases**: 1971 acute DF, DHF, and DSS cases -all patients admitted to the Tan Tock Seng Hospital in Singapore in 2004 - fulfilling WHO 1997 criteria for acute dengue - positive dengue diagnostic tests: - probable dengue:(+)ve acute dengue serology (Dengue Duo IgM & IgG Rapid Strip Test) - confirmed dengue: - positive PCR - no age restriction **Information on dengue history, symptoms, confounders, effect modifiers & comoribities: clinical records** **DM**: clinical records	DM prevalence: Age <60:2% ,Age ≥ 60: 17%
Wieten et al. (2012)	Netherlands (2006-2011)	Tropical and travel-medicine based case series	**Cases**: 132 acute dengue cases -dengue patients serologically tested at Amsterdam Medical Center between 2006-2011 -diagnostic criteria: serological confirmation:positive anti dengue IgM or at least fourfold increase in dengue specific IgG if possible based on one sample from the initial phase and one from the convalescent phase or else according to WHO 2009 criteria for a single sample; clinical picture of probable dengue (WHO 1997 and 2009 criteria) or DHF (WHO 1997) or dengue with warning signs (WHO 2009); **Information on dengue history, symptoms, confounders, effect modifiers & comoribities: clinical records** **DM**: clinical records	DM prevalence: 8%
Sam et al (2013)	Malaysia (2006-2007)	Hospital-based	**Cases**: 10 acute DF/DHF/DSS patients, fatal -fatal cases at the University Malaya Medical Center 2006-2007 -diagnostic criteria: laboratory confirmation: acute phase dengue-specific IgM and IgG; RT-PCR; disease severity classification WHO 1997 ; age range: 11-59; **Information on dengue history, symptoms, confounders, effect modifiers & comoribities:** clinical records **DM**: clinical records	DM prevalence: 30% (pre-existing) Plasma leakage prevalence: 78% of all cases (67% among diabetic patients)

Lahiri et al (2008)	Singapore (2004-2005)	Hospital-based	**Cases**: 9 acute dengue cases, fatal -fatal cases of a total of 1235 admissions with acute dengue in 2004-2005 -all 9 patients had positive laboratory test for dengue (7 by IgM, 3 by PCR) -all 9 patients had evidence for capillary leakage and hemorrhagic manifestations -7 of 9 patients strictly met DHF WHO 1997 criteria - age range: 37-71 **Information on dengue history, symptoms, confounders, effect modifiers & comoribities**: clinical records **DM**: clinical records	DM prevalence: 78% Plasma leakage prevalence: 28% of DM patients
Hasanat et al.(2010)	Bangladesh (2009)	Hospital-based (prospective)	**Cases**: 133 acute dengue cases -patients admitted to Samoritha Hospital in Dakha for dengue - laboratory confirmation by anti-dengue antibody test 1 week of onset of illness -further diagnostic criteria not provided **Information on dengue history, symptoms, confounders, effect modifiers & comorbities:** interviews **DM**: Oral glucose tolerance testing in consenting cases: 1st test between day 3 and 10 of admission and 2nd test before discharge	Status on 1st OGTT (n=133): Normal:25% , Glucose intolerant:54% ,DM:21% Status on 2nd OGTT (n=40): Normal:83% Glucose intolerant:17% Repeatedly normal:25%, Repeatedly abnormal:18% Reverting to normal:55%

Supporting Information

S1 Table: PRISMA Checklist

Chapter 5: Interactions between helminthic infections and diabetes mellitus: Systematic Review

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5.1 Abstract

Background: The hygiene hypothesis states that the lack of exposure natural infections in early childhood increases the chance for the development of chronic allergic and autoimmune diseases. The dual burden diseases (DBD) of infectious (helminthic) diseases and chronic diseases such as diabetes mellitus is high in low- and middle income countries (LMIC). This review aims to summarize the epidemiological and immunological evidence on an association of helminthic infections and diabetes mellitus.

Methods: A systematic literature review was conducted using the MEDLINE database.

Results: Eleven cross-sectional studies and one population based cohort study were identified The prevalence of helminth infections among diabetes and non-diabetic patients or the prevalence of diabetes mellitus among helminth infected and non-infected groups were compared. The results from revealed that helminthic infections and diabetes mellitus are inversely associated.

Conclusion The elucidation of the immune mechanism and epidemiological findings by helminth infections against diabetes mellitus could not only lead to the identification of potential helminth related products but also in indicating a specific cell or molecule for anti-inflammatory induction in diabetes mellitus. Yet, successful mass-deworming programs that decrease the incidence of helminthic infections might have an impact on the incidence of autoimmune mediated disorders including diabetes mellitus in the future. It is of importance to closely follow-up this issue as helminth control activities precede in many LMIC.

Keywords: helminth infections, diabetes mellitus, epidemiology, systematic review

5.2 Introduction

Understanding the complexity of the dual burden of diseases (DBD) between infectious and chronic diseases is a highly relevant issue in low- and middle-income countries (LMIC) as they are particularly frequent in these settings. As of 2015, an estimated 415 million people were affected by diabetes mellitus (DM) worldwide; Type 2 DM making up about 90% of the cases representing 8.3% of the adults global population. In that year, DM resulted in an estimated 1.5 to 5.1 million deaths worldwide corresponding to the 8th leading cause of death globally. The number of people with DM is expected to rise to 592 million by 2035 (WHOa, 2014). Even though the incidence of type 1 DM varies substantially between different populations, it is globally rapidly rising.

Helminthic infections are still a major contributor to the global burden of infectious diseases. Today, more than 1.5 billion people or 24% of the world's population are infected with one or several species of soil-transmitted helminths (STHs). 200 million people are infected worldwide with schistosomiasis and an estimated 120 million cases of lymphatic filariasis (LF) are counted (Global burden of study, 2014). Moreover, according to the World Health Organisation (WHO), countries of East and Southeast Asian, central and south America have high prevalence rates of foodborne trematode infections; An estimated 40 million people have trematode infections (WHO, 2014b) that are accumulating in patients over lifetime and are particularly prevalent in older age groups.

In 1989, the hygiene hypothesis was first formulated by Dr Strachan (Okada et al., 2010). It states that in early childhood infections may protect against allergic diseases at a later age and that the increasing cleanliness of household environments would explain in part the rises in allergic diseases and immune mediated diseases. Over the last decades, autoimmune disorders including DM, have been increasing in geographical distribution (west-east or north-south gradient) (Kondrashova et al., 2005). Reasons mentioned were migration (Jayaweera, 2011), decreasing the infectious diseases (Droste et al., 2000), the low socio-economic related factors (Okada et al., 2010), gene related issues (Tuomi et al., 2014) and environmental factors such as air pollution (Eze et al., 2015) or toxin (Sharp, 2009).

Generally helminthic infections induce a shift from Th1 to Th2 phenotype, accelerate T regulatory and B regulatory phenotypes, and attenuate the levels of the pro-inflammatory cytokines (Bashi et al., 2015, Van Riet et al., 2007). The cellular response to *Ascaris lumbricoides* infection is characterised by a polarized Th2 response with the prominent production of both IL-4 and IL-5, indicated in an Ecuadorian case-control study (Cooper et

al., 2000). The *Trichuris trichiura* infection suggests a mixed cytokine response producing IL-10 (97%), TNF-α (93%), and IFN-γ (32%) while IL-4 (7%), IL-9 (5%), and IL-13 (17%) in Cameroon study (Faulkner et al., 2002). Like other intestinal helminths, hookworm infection is also specified by antibody responses dominated by IgG1, IgG4, and IgE, under the control of Th2 cytokines, predominately IL-4, IL-5, and IL-13, with IL-4 and eosinophil production, while Th1 cytokines such as gamma interferon inhibit its production(Loukas and Prociv, 2001). In Kenyan schistosomiasis studies, pro-inflammatory responses with elevated level of TNF and IFN-γ were shown in the patients (Mwatha et al., 1998), and additional higher IL-13 in Brazilian study (Alves Oliveira et al., 2006). Recently, alternatively activated macrophages (AAM) as a key player in controlling the immune pathology in schistosomiasis has also been highlighted (Barron and Wynn, 2011). The upregulation of Th1, Th17 cells and antibody responses are observed in filariasis infected patients (Babu et al., 2009). In pathogenesis of onchocerciasis, Th2 mediated Treg and IL-13 have a significant character and is related to IL-13 gene mutation which is responsible for allergic hyper-reactivity in the body (Hoerauf et al., 2002, Satoguina et al., 2002).

In the context of type 1 DM, the influence of helminth infections on the onset of autoimmune DM was studied experimentally in non-obese diabetic (NOD) murine models. NOD mice were infected with *Schistosoma mansoni* antigen (Cooke, 2009), *Trichinella spiralis* (Saunders et al., 2007), *Heligmosomoides polygyrus* (Liu et al., 2010), *Litomosoides sigmodontis* (Hubner et al., 2011) and *Dirofilaria immitis* (Imai et al., 2001). The exposures to helminths were found to prevent the onset of type 1 through protection from Th1-mediated β cell destruction (Bashi et al., 2015). Observed alterations in the immune system in response to helminthic antigens involved several types of immune cells including B cells, tolerogenic dendritic cells (DCs), AAM and innate lymphoid cells (ILCs). They all induced Th2, natural killer T cells (NKT) and Treg generation or expansion. The cytokine secretion evoked from NKT, Th2 and Tregs feeds back and maintains the tolerogenic and alternatively activated phenotypes of antigen presenting cells. As a result, diabetogenic (Th1) cells become regulated resulting in only a non-destructive insulitis (Zaccone and Cooke, 2013).

Obesity, type 2 DM and cardiovascular diseases share a metabolic profile characterized by insulin resistance (IR) and chronic sub-acute inflammation (Donath and Shoelson, 2011). The insulin resistance, a predominant characteristic of type 2 DM and related forms (e.g. Latent Autoimmune Diabetes of Adults LADA) is strongly linked to obesity and specifically to visceral fat mass and therefore to obesity-associated metabolic

inflammation rather than to stimuli of infections. The inflammatory signalling can also be stimulated by cellular stress such as reactive oxygen species, endoplasmic reticulum stress, hypoxia and lipotoxicity, free fatty acids which can all be enhanced in the obese induced insulin-resistant state. Then, chronic helminthic infections, as an inducer of type 2 immune responses induce IL-4, IL-5, IL-13 and eosinophilia production which further boosting the alternatively activated macrophages (aaM) development together with regulatory T and B cells and the production of anti-inflammatory cytokines like IL-10 and TGFβ, leading to insulin sensitivity (Allen and Maizels, 2011, Hubner et al., 2013), which was shown in diet induced obesity mice studies by *S. mansoni* infections (Hussaarts et al., 2015). The helminth mediated protective mechanism against type 2 DM is that the helminth induced type 2 immune responses increase IL-4 production by eosinophil and aaM polarization in the adipocytes, further suppresses the activation of classically activated macrophages leading to anti-inflammatory immune responses, thus improving insulin sensitivity and inhibiting β-islet cell destruction (Donath et al., 2008) and the study conducted on Flores Island in Indonesia, showed that individuals infected with STH had a lower insulin resistance (Wiria et al., 2012). Many studies provide some evidence that helminth induced immunomodulation mechanism shield the onset of type 1 DM. However, the mechanism of interaction of helminths on type 2 DM is still unclear and need to be further investigated. It is also important to keep in mind that the extent of helminth related immunomodulation varies with helminths species, intensity of the infection and the direct metabolic reaction of host's immune system.

The objective of this review was to evaluate the epidemiological evidence related to an association between helminthic infections and the development of DM and the interaction of two diseases.

5.3 Methods

Search strategy

A systematic literature review using MEDLINE database was performed to identify articles in which an association between helminthic infections and DM was examined. The following search terms (MeSH terms) were: Trematodes infection (dicrocoeliasis, echinostomiasis, fascioliasis, fascioloidiasis, opisthorchiasis, paragonimiasis, schistosomiasis, schistosomiasis haematobium, schistosomiasis japonicum, schistosomiasis mansoni); Cestodes infections (diphyllobothriasis, sparganosis, echinococcosis, hymenolepiasis, taeniasis, cysticercosis); Nematodes infections (larva migrans, ascariasis, trichuriasis, oxyuriasis, strongyloidiasis); "helminth", “helminthiasis", "parasitology" in combination with

"diabetes mellitus", "diabetes, type 1", "diabetes mellitus, type 2", "insulin resistance", "IDDM", "NIDDM" alone and in combination.

Inclusion and exclusion criteria

We included all articles without restriction on language, publication date (up to 31 August 2016), study settings, design or age of study participants and also articles describing the epidemiology, clinical signs, and laboratory parameters of either helminth infected or diabetic patients, and the assessment of complications. The studies conducted in animals settings were excluded in the review. The PRISMA criteria (PRISMA and Moher, 2009) were used for validity assessment of the published articles.

Data extraction

The following information was extracted from the publications: Year of the study, year of publication, place (country) of study, study design, study populations and their characteristics, study definitions and diagnostic criteria for DM and helminth infections and adjusted confounder. Moreover, we extracted data on the prevalence of DM among helminth infected persons and *verse versa* and the reported estimates of the association such as adjusted and unadjusted odd ratios (OR) with 95% confidence intervals (CI). The transition process for this review is confidential and anonymous. The two reviewers independently extracted the data from the published manuscripts and disagreements were sorted out by discussion.

5.4 Results

Our literature search revealed 52,958 articles (Fig. 1) of whom 34,691 articles were animal studies and were excluded. Among the remaining 18,267 articles, 18,255 articles were not relevant (clinical single case reports, non-comorbid studies, and overview of helminthic infections in a specific countries or particular geographical settings, and cellular or molecular study of helminthic infections) and were not considered for the review. Finally twelve articles which reported the relationship between helminthic infections and either type 1 or type 2 DM, were retained (Table 1).

Eleven studies had a cross-sectional and one study had a cohort design. They were conducted among the communities in countries endemic for different helminth species. In the articles, the prevalence of helminthic infections was reported among diabetic, pre-DM and non-diabetic subgroups and the association assessed. The epidemiological studies in this review also described the prevalence of DM in helminth infected versus non-infected groups and means of measures to describe its association. In Table 1 the key features of the identified studies are summarized. Below a short description of each study is provided.

Aravindhan and colleagues conducted two studies in India in which they observed a lower prevalence of lymphatic filariasis (LF) in patients with type 1 and type 2 DM compared to persons with a normal glucose tolerance (NGT). The study participants were recruited from the Chennai Urban Rural Epidemiology Study (CURES). The first study identified type 1 DM patients (n=200), selected healthy NGT persons from the CURES and compared their seropositivity of LF (Aravindhan et al., 2010b). Type 1 DM was diagnosed by C-peptide assay < 0.3 pg/mL and GAD–specific autoantibody levels ≥10 IU/mL. Bancroftian LF was detected in the serum samples analyzed by using the *Wuchereria bancrofti* Og4C3 antigen-capture ELISA. The antibody titer to LF was determined by the serum antibody (IgG and IgG4) levels against *Brugia malayi* antigen. The prevalence of LF was 0% in type 1 DM persons and 2.6% among NGT persons (p = 0.03). The blood parameter also supported these findings that *Brughia malayi* antigen specific IgG4 which is a standard marker of present infection, was lower among type 1 DM patients. However, another indicator for *Wuchereria bancrofti* exposure, the levels of antigen specific IgG, did not show any difference between the groups. In this study, the authors argued that socioeconomic factors were unlikely to be a confounding factor among type 1 DM and NGT persons after stratification.

In the second study, Aravindhan and colleagues observed a significantly lower prevalence of LF among type 2 DM subjects (5.7% in both newly diagnosed and 4.3% in those under treatment) compared to pre-diabetic subjects 9.1% and non-diabetic subjects 10.4% (Aravindhan et al., 2010a). There was also a lower significance level of filarial antigen loads as well as serum cytokine levels of the pro-inflammatory cytokines (especially IL-6 and GM-CSF and TNF-α) in LF positive diabetic subjects compared to those who were LF negative. The levels of anti-inflammatory cytokines such as IL-10, IL-13 and TGF-β were not significantly different among the study groups. The study concluded that the LF modulated inflammatory reaction supressed the pro-inflammatory responses in the body and provided a protective mechanism against type 2 DM.

Concerning *Enterobius vermicularis* infection, a large, population-based cohort study (Bager et al., 2012) was conducted to determine the association between childhood *E. vermicularis* infection and incidence of chronic inflammatory diseases such as asthma, juvenile arthritis, ulcerative colitis, or Crohn's disease, including type 1 DM in the children who were born in Denmark between 1995 and 2008 and registered in the Civil Registration System. The results revealed that childhood enterobiasis did not reduce the risk of having type 1 DM. An incidence rate ratio of 1.05 (95% CI: 0.79 –1.12). Similar results were obtained for

the other chronic diseases. The research participants were identified from in-patient or outpatient hospital diagnosis record of chronic inflammatory disease from National Patient Registry and history of medication with mebendazole. The authors mentioned some possible logic with regards to the findings that general practitioners overprescribed of anthelminthic drugs for other helminthic infections which could diminish the effect of *E. vermicularis* on chronic diseases On the other hand, the results could also be due to the nature of enterobiasis infection which is only persistent for a short time period and not repeated. Hence insufficient to modulate the immune system such as other helminths infections as *Ascaris* or *Trichuris* infections may do.

In a study in Egypt with a cross-sectional design the prevalence of the Islet cell antibody (ICA) seropositivity was two times higher among diabetic children (50%) than the children with schistosomiasis infection (25%). In addition, normal glucose tolerance or lower IR to intravenous glucose load was also found in the children with schistosomiasis compared to the other two groups. Gender, being a sibling, previous medical illness, and the blood results for liver and kidney functions did not show significant difference between the groups. The findings suggested that the autoimmune reaction of the children with schistosomiasis infection could lessen islet cell dysfunction, resulting decreased risk of developing glucose intolerance and DM in future (Soliman et al., 1996).

Similarly, in rural China, Chen and colleagues (Chen et al., 2012) performed a cross-sectional study which reported the association of the previous schistosome infection (PSI) and the presence of DM and metabolic syndrome among individuals aged 60 years and older. The researchers found a strong protective effect of PSI on current DM status (aOR 0.51, 95% CI 0.34–0.77). Five different statistical models were used to explore the association. In four models, the associated was adjusted for a series of other factors such as age, sex, body mass index (BMI), physical activity, family history of DM and educational levels but also on metabolic markers such as plasma fasting blood glucose (FBG) and postprandial blood glucose (PBG), haemoglobin, haematocrit, and eosinophils, serum insulin, liver function tests, lipid profile, HbA1c levels. The different models gave consistent and statistically significant results. The ORs ranged from 0.44-0.52. PSI was reported by the study participants and confirmed by using hospital records. DM was defined according to WHO 1999 criteria. Furthermore, the study found a protective effect of PSI against metabolic syndrome (aOR 0.40, 95% CI 0.27–0.58) by using the same five models. However, there were some potentially important confounding factors such as the age of study participants, the

consumption of alcohol, smoking status and self-reported physical activities were not able to be considered in the study.

In Brazil, Mendonca and colleagues carried out a cross-sectional study (Mendonça et al., 2006) to determine the frequency of positive *Strongyloides stercoralis* serology in DM patients compared to healthy controls. They found that *S. stercoralis* seropositivity in the DM group (23%) was significantly higher (OR 3.9, 95% CI 1.6-15.9) then in the control group (7.1%). In this study, the diagnosis of strongyloidiasis was made by the detection of larvae in the stool as well as serodiagnostic test (ELISA) using the filariform larvae antigen. The study provided a positive association between *S. stercoralis* serology and DM and concluded that the *S. stercoralis* infection superimposes and put the DM patients at fatality risk, especially those with poor sugar control.

Another cross-sectional survey conducted among aboriginal adults living in a remote community in northern Australia (Hays et al., 2015) was done to assess the relationship between infection with *S. stercoralis* and the likelihood of having type 2 DM. The diagnosis of DM was defined by HbA1c testing or being under DM treatment in the past. The serological diagnosis for *S. stercoralis* was performed by using ELISA. The study showed that 36% of participants showed prior infection with *S. stercoralis* and 51% of them had type 2 DM. Those with previous *S. stercoralis* infection were 61% less likely to have a diagnosis of type 2 DM than those uninfected (OR 0.39, 0.23–0.67, P = 0.001). 51% in ≥ 0.2 value level OR 0.49, 95% CI 0.30-0.83, p = 0.007 and 57% in ≥ 0.4 cut-off value (OR 0.43, 95% CI 0.25-0.75, p = 0.003) after adjusted for age, triglycerides, systolic blood pressure and BMI using a propensity score. These findings indicated that a *S. stercoralis* infection seemed to reduce the risk for a type 2 DM in adults although in the study community the prevalence was very high in all individuals, including the DM patients.

To assess the predisposition of parasitic infections among DM population, Nazligul and colleagues conducted a cross-sectional study in southeastern Anatolia, Turkey (Nazligul et al., 2001). Collected fresh stool samples were investigated macroscopically and microscopically by native, lugol, and flotation methods. The study showed that non-DM participants had a higher prevalence of intestinal parasite infection compared to DM patients (55.9% vs. 47%). The prevalence rates of intestinal parasitic infections in DM patients were high for *A. lumbricoides* (57.5%) followed by *T. trichiura* (14.2%), *Entamoeaba histolytica* (13.3%), *Giardia intestinalis* (11.7%), and *Taenia saginata* or *Hymenolepis nana* (3.3%). Ascariasis was the most common parasitic infections in both groups. No statistically

significant differences were reported between the prevalence of the parasites and age groups as well as gender.

To explore the prevalence of acquiring helminth infections and its associated risk factors in the DM patients, a cross-sectional study was done in Nigeria by Akibo and colleagues (Akinbo et al., 2013). With an overall prevalence of 18.7% of intestinal parasitic infections, however, none of non DM participants were infected. The study also showed DM status was significantly associated with intestinal parasitic infections (OR 14.19; 95% CI: 0.842-239.22; p=0.022). The most frequent parasites were *A. lumbricoides*, hookworm, followed by *E. histolytica* among DM patients. The prevalence of infections was only significantly influenced by age groups and types of toilet used but not by the other variables. The types and duration of DM status don't reveal any significant effect on the prevalence of infections.

Bafghi and colleagues carried out a descriptive cross-sectional study among Iranian DM and non-DM participants to determine the frequency distribution of intestinal parasitic infections in diabetic patients (Fattahi Bafghi et al., 2015). The study indicated the intestinal parasites rate in diabetic patients was slightly higher compared to control group (24.4% *versus* 23.2%). The most common parasites found in both groups were protozoa (*G. intestinalis, Entamoeba coli, Cryptosporidium* sp*., Blastocystis hominis)* and *A. lumbricoides.* In this article, the diagnostic methods of the parasitological examination were not clearly stated. Due to higher prevalence of protozoa infection, the author argued that even though the protozoa detection is not usually included in the routine stool examination, the standard diagnostic methods of protozoan detection should be considered especially for immunocompromised patients like DM.

A similar cross-sectional study exploring the intestinal parasite prevalence rate of DM patients was performed in Sohag University hospital, Egypt (Elnadi et al., 2015). The stool smears were prepared with different standard methods of concentration for microscopy examination and blood HbA1c assessment was done to detect the DM status of research participants. The prevalence of intestinal parasites was 25% in diabetic group and 7% in healthy controls. There was a high prevalence rate in age group of less than 10 years old children, marked lower rate in 10-30 age groups and with a gradually increasing rate with ages, a highest prevalence rate was seen in 50 - 59 years old age group. The protozoa infections (*G. intestinalis*, *E. histolytica* and *E. coli*) with cestode infections (*H. nana*) were mostly diagnosed. Compared to type 1 and type 2 DM, 52% in type 1 compared to 16% with

type 2 were reported with a significant association (X^2:11.1, p<0.001) between types of DM and intestinal parasites prevalence with type 1.The study also described that uncontrolled DM status was a risk factor for having higher prevalence rate of parasites and significant relationship in both type 1(p<0.007) and type 2 (p<0.01).

Similarly, with a total cases of 150 diabetic patients and healthy control group of 85 non-diabetic individuals were recruited to conduct a hospital based cross sectional study in Limbe and Buea Municipalities of Cameroon (Tangi FB, 2016). The study also aimed to examine the prevalence and type of intestinal parasites among DM patients. The study showed the overall prevalence of intestinal parasites among diabetics was lower than the control group (10% vs 23.5%) with a protective association between intestinal parasitic infections and diabetic status (OR: 0.36; 95% CI: 0.17-0.75; p=0.005). The detected parasites were mostly *E. histolytica* along with *B. hominis*, *A. lumbricoides,* hookworm and *C. parvum.*

The Table 2 summarizes the outcomes of the studies on DM in relation to different types of helminthic infections and the referred animal models. The concepts of conducting the epidemiological studies included in this review are based on animal models when NOD mice were infected with either *S. mansoni* or injection of soluble egg antigen (SEA) or soluble worm antigen (SWA) from this helminth species where the protection mechanism through immunomodulation against the DM especially type 1 has been clearly shown.

5.5 Discussion

This systematic review summarizes the current knowledge and epidemiological evidence, immunological and metabolic interaction of DM associated with different types of helminths. Most studies in this review confirm a negative association between helminthic infestations and DM. or they demonstrate that helminthic exposure contributes to a protective effect to the population from having DM either type 1 or type 2 (Aravindhan et al., 2010a, Aravindhan et al., 2010b, Soliman et al., 1996, Chen et al., 2012, Hays et al., 2015, Tangi FB, 2016, Nazligul et al., 2001). However, some other studies indicate that helminth infection may increase the severity of DM (Mendonça et al., 2006, Fattahi Bafghi et al., 2015, Akinbo et al., 2013, Elnadi et al., 2015). The Danish study did not support the fact that common childhood enterobiasis infection protected against chronic inflammatory diseases (Bager et al., 2012).

The strengths of this systematic review are firstly, it highlights the importance of complex helminthic interactions and immune modulations in chronic diseases like DM. Second, most of the studies were community based which a large number of research participants were recruited to avoid bias and the confounder adjustments such as age and sex

were carefully considered in the studies. Third, clear case definitions with laboratory and biochemical investigations of DM and helminthic infections and measures of association were well described in the studies. However, there are also limitations. This review is very confined to the published articles; the results of studies couldn't identify the direct causal link between helminthic exposures and DM due to cross-sectional study design; poorly controlled of some unmeasured confounding factors in a few studies. Nevertheless, this review draws an attention on the complex immune mechanism between two diseases and any possible solutions or potential therapeutic agents to be considered.

As helminths are also known for reducing energy overload of the body by taking up the nutrition and anti-inflammatory response, they protect the individuals from having atherosclerosis formation in the body. A study in Indonesia evidence that helminthic infections are negatively associated with the risk factors for atherosclerosis and cardiovascular disease, mediated by an effect on carotid media thickness, lipid levels, BMI and waist-hip circumference (Wiria et al., 2013). This finding is supported by another study that the individuals infected with chronic *Opisthorchis felineus* had lower levels of serum cholesterol and attenuated the atherosclerosis development (Magen et al., 2013). One step moving forward, that is pure, non-pathogenic helminth derived molecules or immunomodulators is another choice to avoid the pathological consequences of helminths. There are a number of therapeutic studies in which the different types of immunomodulators were used in different diseases in mouse models (Schnoeller et al., 2008, Bashi et al., 2015) but they have not yet been used in human beings because the interaction of immune system between mice and human are quite different and complicated.

In past few years, a large amount of studies or clinical trials have been conducted to find out the possible drugs or alternative solution for treatment of DM. The studies have shown that chronic tissues inflammation is a critical factor in immune mediated diseases and anti-inflammatory drugs/therapy improves systematic insulin sensitivity in type 2 DM patients by reducing 0.37% of HbA1c level after 48 weeks (Goldfine et al., 2013) and in another study, treating the rheumatoid arthritis patients with anti TNF-α upgrades the insulin sensitivity (Esser et al., 2015). However, due to complicated inflammatory signals in insulin sensitivity, new diabetic therapies emphasizing on the specific inflammatory cytokines or receptors are still needed to have an effective clinical significance in type 2 DM patients. A substitute like probiotics has also been proved in many evidence based immune related research. The probiotic supplementation (*Lactobacillus* strains) in pregnancy and early life

relieve atopic sensitivity in infants (Elazab et al., 2013) and using probiotics in the first 6 months of life decreases type 1 DM-associated autoantibodies in the children who have genetically high risk and only 2% prevalence at 24 months of age in the PRODIA study (Ljungberg et al., 2006) but the detailed immune mechanism of the probiotics is still debateable. Furthermore, there is evidence that the gut microbiota also shows a significant part in the regulation of glucose and lipid metabolism. Many studies in both animal and human models suggested that changes in the composition and metabolic function of the obese microbiota in obese individuals compared to lean controls extract more energy from the diet and interacts with host epithelial cells, leading to increased storage of fats and hepatic triglycerides leading to increased liver lipogenesis, hepatic insulin resistance and hyperinsulinaemia (Turnbaugh et al., 2006, Hartstra et al., 2015). As the microbiome and the helminths have the same characteristics in terms of interplaying with the nutrition and modulating the host immune system (Glendinning et al., 2014), micorbiome could also be an alternative option for the therapy of obesity and metabolic diseases including type 2 DM. Additional research with a proper understanding of environmental influences on the microbiota and the consequences of functional changes within microbiota on metabo-inflammatory diseases and the impact on its host health are crucial.

There is another interesting facet, namely the “fetal origins” hypothesis stating that a mother's metabolism during pregnancy which is influenced by maternal nutrition, infection status and underlying social factors such as maternal smoking determine the baby's size and increase risk of DM in childhood and early adult life (Harder et al., 2001). Many epidemiological studies have demonstrated the relationship between low birth weight and the later development of insulin resistance, type 2 DM, hypertension, hyperlipidaemia and cardiovascular diseases in both men and women across Europe, Asia, Africa and the USA (Newsome et al., 2003). This hypothesis could be applied assuming that if a pregnant mother has inadequate nutrition due to chronic helminthic infections, the offspring could have a lower weight at birth and a higher chance of getting insulin resistance or type 2 DM in adult life and this aspect of study is worthwhile to explore any possible association between maternal infectious status and its impact on health outcome of newborn for evidence based purpose.

Nowadays, large-scale deworming programs are being introduced into tropical helminth endemic countries aiming to reduce the helminth related morbidity mainly for STH and to have a global elimination of a certain helminthic infection such as LF and schistosomiasis. This initiation of mass anthelminthic therapy is universally acceptable and

cost-effectiveness in controlling the helminth infections, however, on the other hand, it might produce an unwanted effect on immune-metabolism of the human beings. There is a cluster-randomized placebo controlled trial in Indonesia examining the association of the helminthic infections with the insulin sensitivity and the protection mechanism of helminths are possibly diminished by anthelminthic treatment (Tahapary et al., 2015). The finding of this study is very relevant and important to the all helminth endemic countries which are currently under mass deworming programs.

In summary, there are controversial scientific associations between helminth infections and DM status and different prevalence rates of parasitic infections among DM population. Additional epidemiological research are still required to identify the knowledge gap in learning which extent of helminthic infections could result how much effect on glucose or insulin metabolism in detail and also their advantages for therapeutic purposes. Community based, well designed with longer follows up or longitudinal studies with assessment of the fluctuations of specific cytokine and inflammatory mediator caused by a particular helminthic type in different country settings would be absolutely vital to identify the causal relationship between the helminthic infections and insulin sensitivity in type 2 DM.

5.6 Conclusion

This systematic review discovers the comprehensive updates of immunological and epidemiological interactions between helminths and DM covering from basic immuno-cellular level to extensive metabolic pathways and the evidence of potential helminthic derived therapies is also thoroughly discussed. Aside from the divided findings of relationship between helminth infections and DM condition among the articles, in a case if helminthic infections regulate mechanisms of immune system in a good way, the administration of helminth derived immunomodulators or substitutes could still be a critical challenge in the treatment of DM due to the undesirable and pathogenic virulence effect of the helminths. On the other hand, the health care policy makers in the helminth endemic countries should be well prepared for the possible uprising of DM at a later time due to the consequences of deworming.

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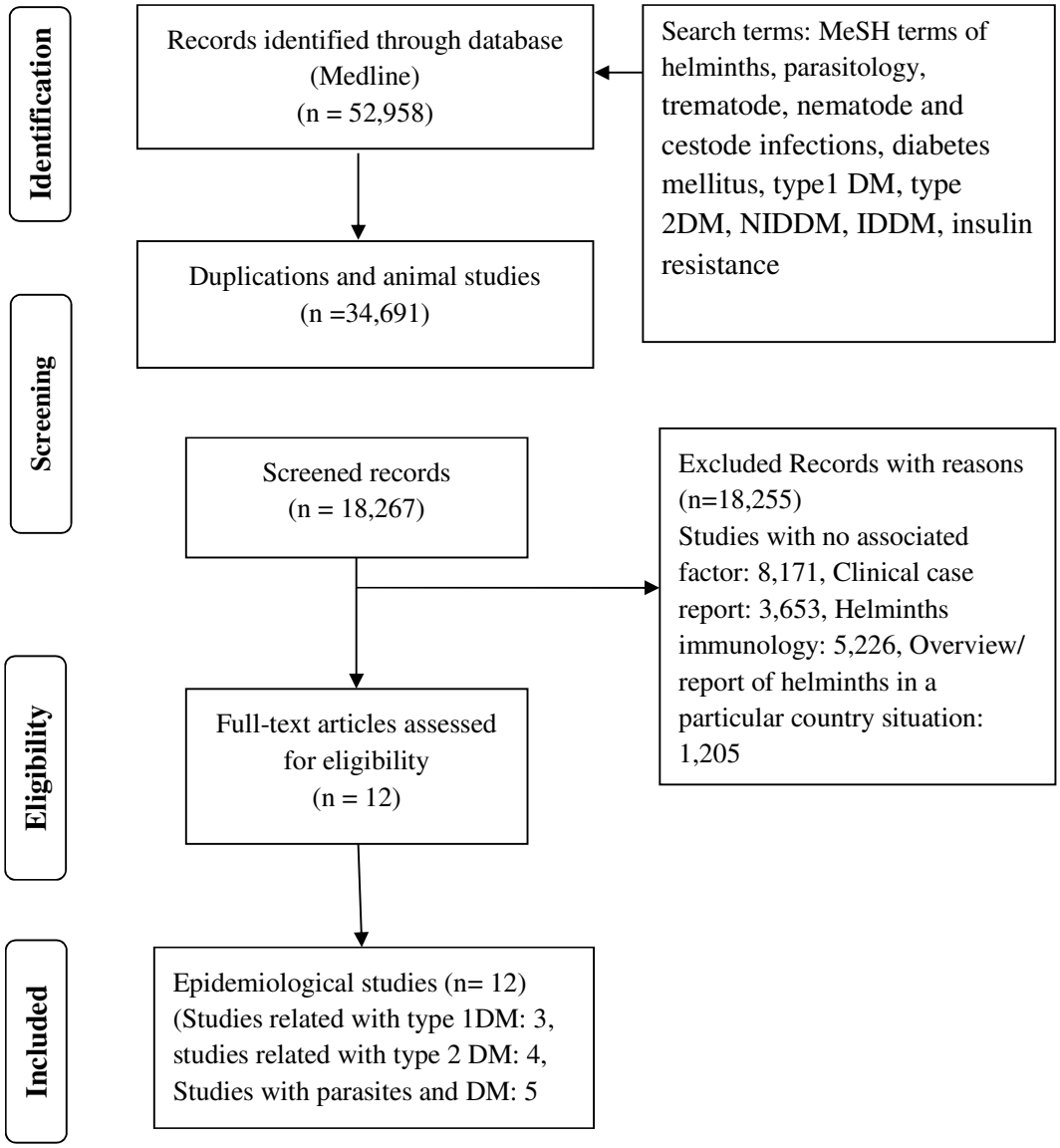

Figure 1: PRISMA flow diagram of helminths and diabetes mellitus

Table 1. Summary of the epidemiological studies of helminths and diabetes mellitus

Year of publication and first Author	Study design and setting	Characteristics of the study population	Measures of association: Odds ratio (OR) with 95% confidence intervals (CI) and p value)
Hays et al (2015)	Cross-sectional study in Australia	259 Aboriginal adults living in a remote community in northern Australia Data extraction: age, sex, date of testing, S. stercoralis ELISA titre, haemoglobin, total eosinophil count, percentage eosinophilia, height, weight, calculated BMI, diabetic status and HbA1 C Triglyceride level, HDL and total cholesterol. **Diabetes**: HbA1c ≥ 6.5% or greater, or RBS ≥ 11.1 mmol/l, or FBS ≥ 7.0 mmol/l, or patients with past medical history of receiving diabetes treatment **S. stercoralis serology testing**: by ELIZA (The reference values: 0.3 units of absorbance)	92(36%): S. stercoralis infection 131 (51%): T2DM. aOR = 0.39, 0.23–0.67, P = 0.001 (Those with previous S. stercoralis infection were 61% less likely to have T2DM than those uninfected, adjusted for age, triglycerides, blood pressure and BMI using propensity score)
Chen et al. (2012)	Cross-sectional population based study in Jiading County, Shanghai, China	9939 individuals, both genders, ≥40 years and above **Data collection** Socio-demographics, medical history, and lifestyle factors, Weight, height, waist circumference, blood pressure, intensity, duration, and frequency of physical activity using International Physical Activity Questionnaire, separate metabolic equivalent hours per week Blood samples at 0 and 2 hours after 75-g oral glucose tolerance test using the glucose oxidase method, FBG,PBG Haematological parameters including haemoglobin,haematocrit, and eosinophils. Serum insulin, ALT, GGT, albumin, lipid profile, serum insulin concentration HbA1c level **Previous *Schistosoma* Infection:** self-reported medical history and validated by cross-referencing with the registry data generated during screening program in 1989	**Prevalence of PSI:** 0.05%in 40-49, 1.10% in 50-59, 8.89%in 60-69, 17.85% in 70 years and above **Prevalence of diabetes:** 18.04% the entire population and 24.25% in individuals aged 60 years and older (more than 53% newly diagnosed diabetes) **Prevalence of DM and metabolic syndrome in PSI group** (14.9% vs 25.4%, *P* < .0001) compared with the non-PSI group (14.0% vs 35.0%, P < 0.0001). Adjustment for insulin resistance: adjusted OR 0.51, 95% CI 0.34–0.77, *P* = 0.0012 for diabetes; adjusted OR 0.40, 95% CI 0.27–0.58, *P* < .0001 for metabolic syndrome

		DM diagnosis (WHO 1999 criteria): (1) FBG ≥7.0 mmol/L or (2) PBG ≥11.1 mmol/L or (3) self-reported diagnosis of diabetes by physicians and use of antidiabetic medications. **Metabolic syndrome**: National Cholesterol Education Program Adult Treatment Panel III criteria; any 3 or more of the following conditions: (1) BP 130/85 mmHg or greater or taking antihypertensive drugs; (2) WC 102 cm or greater in men and 88 cm or greater in women; (3) TG 1.69 mmol/L or greater; (4) HDL-C less than 1.03 mmol/L in men and less than 1.29 mmol/L in women; (5) FBG 6.1 mmol/L or greater or taking hypoglycemic medications. **Insulin resistance:** homeostasis model assessment of insulin resistance (HOMA-IR) higher than 2.50.	
Aravindhan et al (2010)	Cross-sectional in Southern India	Study subjects from ongoing CURES epidemiological study, every 10th individual in Phase 1 participated (26,001 individuals from urban component of CURES). Details of the study participants using questionnaires and form 4 groups by randomly selected. Group 1- 943 NGT Group 2- 154 IGT Group 3- 158 NDDM Group 4- 161 KDM Anthropometric measurements (height, weight, and waist circumference, BMI, fasting plasma glucose, lipid profile and HbA1C by using standardized techniques. **DM diagnosis:** HbA1c level measurement **Detection of Bancroftian LF** antigen levels by *Wuchereria bancrofti* Og4C3 antigen-capture enzyme linked immunosorbent assay **Determination of anti-filarial antibody titre (IgG and IgG4)** against *Brugia malayi* antigen (BmA) by ELISA **Determination of serum cytokine levels** by using a Bioplex multiplex cytokine assay system	**Prevalence of LF:** 10.4% in the NGT, 9.1% in IGT, 5.7% in ND-DM and 4.3% in KDM. The differences in the prevalence rate between NGT and KDM (p= 0.0463) and NGT and ND-DM (p = 0.0095) were significant. **Geometric mean of CFA levels:** NGT-1,594 (127–32,768), IGT-1,520 (209–16,345), NDDM-929 (129–32,768) and KDM-351 (163–1,126) with the differences in the antigen levels between the KDM and the NGT (p= 0.04) and the KDM and the IGT (p = 0.04) The **mean IgG levels:** lower in the KDM compared to NGT (p=0.0023). In comparison to controls (DM^-LF^-), the diabetes only (DM^+LF^-) group had high levels of IL-6 and GM-CSF; (p=0.01) In DM^+LF^+subjects, there was a significant reduction in the levels of IL-6 (p=0.05)and GM-CSF(p=0.05) compared to the diabetic only

			group ($DM^{+}LF^{-}$). IFN-γ levels were significantly elevated in diabetic only group compared to controls. TGF-β was significantly higher in both the diabetic groups compared to controls (p=0.01). No significant difference in IL-10 levels among the three groups.
Mendonc aet al (2006)	Cross-sectional study in Brazil	120 ambulatory patients (28-77 years; 78 type 2 diabetic and 42 healthy controls) from a strongyloidiasis hyperendemic area. Patients and controls with similar background and current socio-economic conditions **DM diagnosis**: Glycated haemoglobin (HbA1c) level ***S. stercoralis* detection*:*** Three faecal samples on consecutive days. Parasitological diagnoses were carried out using Baermann and Hoffmann method. Serology tests by IFAT, ELISA and WB	The frequency of positive *S. stercoralis* serology in diabetes vs controls was 23% vs 7.1% (P < 0.05). OR: 3.9 (CI, 1.6–15.9, P < 0.05).
Aravindhan et al (2010)	Cross sectional study in Southern India	Total 762 (200 type 1 DM; 562 NGT) **Type-1 DM diagnosis:** the presence of lean body weight with hyperglycemia, ketonuria,and absence of insulin release by C-peptide assay <0.3 pg/mL and GAD–specific autoantibody levels ≥10 IU/mL) Controls: serum C-peptide values >0.3 pg/mL and were GAD autoantibody negative (from the Prevention Awareness Counseling and Evaluation project) Medical history, a physical examination, Anthropometric measurements, fasting plasma glucose, serum lipids, and glycated hemoglobin (HbA1C) estimations were obtained. **Detection of Bancroftian LF**: The filarial antigen levels and prevalence were analyzed by the *Wuchereria bancrofti* Og4C3 antigen-capture by	The prevalence of LF was significantly different (p = 0.026) between NGT persons (2.6%) and those with T1DM (0%). Filaria-specific IgG4: lower in the T1DM group (2%) than in the NGT group (14%) (p<0.001)

		ELISA **Determination of antibody titer to LF:** IgG and IgG4 levels against *Brugia malayi* antigen was determined by ELISA as described. IgG titers >14 and IgG4 titers >100 were considered positive.	
Bager et al (2012)	Danish cohort study	A Danish cohort of 924,749 children born from January 1, 1995 through December 31, 2008 using the Civil Registration System, (n=924,749; age 0–14 years); 132,383 of these children (14%) filled a prescription for Mebendazole, 102,482 of the children (11%) had a household peer who was registered with a filled Mebendazole prescription, and the remaining 689,884 children (75%) comprised the reference group. ***Enterobius vermicularis* infection**: Any child with Mebendazole prescription **Diagnosis of asthma, type 1 DM, juvenile arthritis, ulcerative colitis, or Crohn's disease:** from the National Patient Registry.	The incidence rate ratios : Type 1 DM: 1.05 (95% CI: 0.79 –1.12), Juvenile arthritis: 1.13 (95% CI: 0.94 –1.37), Ulcerative colitis : 0.77 (95% CI: 0.41–1.46) Crohn's disease: 1.44 (95% CI: 0.82–2.53) Asthma: 1.07 (95% CI: 1.00 –1.13),
Soliman et al (1996)	Cross-sectional study in Egypt (University of Alexandria Children's Hospital)	94 (14 children with IDDM, 30 of the non-diabetic siblings of patients with IDDM, 40 children with chronic Mansoni schistosomiasis, and 10 normal children) History-taking (age, nutrition, consanguinity of the parents, previous viral infections (measles, mumps, and rubella), and duration of IDDM or schistosomiasis), anthropometric data, height and weight, the stage of sexual maturation recorded according to Tanner's sex maturity rating system. **Schistosoma infection**: Viable schistosoma eggs in their freshly prepared faecal and/or urine samples. **DM diagnosis**: venous blood sample from all the children to measure ICA, blood urea and serum insulin-like growth factor-I, creatinine, alanine transferase, alkaline phosphatase, and bilirubin concentrations, OGTT and 2HPP, estimation of insulin concentrations by radio-	Prevalence of Islet Cell Antibody: 25 % in children with schistosomiasis, 13 % in diabetics' siblings, and 50 % in children with IDDM. OGTT: normal in all the studied children. Five minutes after intravenous infection of glucose load: lower insulin increments in children with schistosomiasis compared to the other two groups. IGF-I concentrations: lower in children with schistosomiasis *vs* children in the other two groups.

		immunometric assay after intravenous injection of glucose	
Bafghi et al (2015)	Cross-sectional study in Yazd, Iran	250 diabetic patients and 250 healthy individuals with cluster random sampling in Yazd diabetes research centers from December 2012 to December 2013 Parasitic infection detection: Stool samples under microscope DM diagnosis: known cases from diabetic research centers	Higher intestinal parasites rate in diabetic patients (61:24.4%) than healthy control group (58:23.2%)
Tangi et al (2016)	Cross-sectional hospital based study in Cameroon	A total of 235 participants in the study **DM diagnosis** Cases: 150 diabetic patients who live in the study area and visit diabetic units in hospitals. Controls: Non-diabetic individuals in the study area (confirmed with random blood sugar level) **Parasitological examination**: Stool samples were analyzed by direct microscopy followed by modified ziehlnelsen method was used to detect intestinal protozoa. Stoll's technique was used for the quantification of helminth eggs	The overall prevalence of intestinal parasites among diabetic patients : 10% (*Entamoeba histolytica* 10(6.7%), *Blastocysti shominis* 4(2.7%), *A. lumbricoides* 1(0.67%), Hookworm 1(0.67%) and *Cryptosporidium parvum* 1(0.67%)). An overall prevalence of intestinal parasites among controls: 23.5% (*Entamoeba histolytica*: 18(21.2%), *A. lumbricoides* 2(2.4%), and *Blastocystishominis* 1(1.2%). A significant protective association with the prevalence of intestinal parasites (OR: 0.36, CI=0.17-0.75; p=0.0051)
Akinbo et al (2013)	Cross-sectional study in Nigeria	A total of 180 individuals were included in this study. DM diagnosis Cases: 150 (41 males and 109 females) DM patients attending clinics Controls: 30 (7 males and 23 females) non-DM individuals Parasitological examination: freshly collected stool samples were analyzed under microscope after preparing stool slides with standard methods. Blood examination: CD4 count with flow cytometry, Hb concentration	An overall prevalence of of intestinal parasitic infections among DM patients: 18.7% DM status was significantly associated with the prevalence of intestinal parasitic infections (OR:14.192; 95% CI :0.842-39.22; p=0.022). Females have higher infection rates (78% vs. 21.4%) Age group (51-60 yrs) and persons who use pit

		with autoanalyzer to assess the status of anemia	latrines have highest prevalence rate of infection. Anemia is also associated with DM patients who had infection (OR:3.310; 95% CI:1.311-8.361;p=0.016)
Elnadi et al (2015)	Cross-sectional hospital based study in Egypt	A total of 200 participants aged 1-70 years (100 from hospital DM outpatient clinic, 100 cross matched controls) Parasitological examination All fecal samples were preserved with 10% formalin and macroscopically and microscopically inspected after preparing by simple sedimentation, simple floatation, formalin-ethyl ether sedimentation, Kato thick smear with modified Ziehl-Neelsen stein DM diagnosis: HbA1c assessment of blood (considered as normal range of 4% -5.9%)	Infection prevalence rate Cases (25%) vs controls (7%) *G. lamblia* (22% vs. 5%) *E. histolytica* (7% vs. 3%) *H. nana* (5% vs. 3%) *E. coli* (8% vs. 3%) *E. hartmanni* (3%), D. *D. Fragilis* (1%), Only in DM group: *C. parvum* (5%) and microsporidia (3%) Type 1 vs. type 2 52% vs. 16% (X^2: 11.1, p<0.001) Uncontrolled vs. controlled (type 1) 40% vs. 26.4% (X^2:7.2,p<0.007) Uncontrolled vs. controlled (type 2) 32% vs. 8% (X^2:5.46,p<0.01)
Nazligul et al (2001)	Cross-sectional study in Sanliurfa province, Turkey	A total of 1224 participants (200 (type 1:16, type 2:184) DM patients and 1024 non-DM subjects)) aged 15-79 years DM diagnosis: 26 with insulin therapy, 106 with oral antidia-betic agents, 21 with diet alone, and 47with no therapy Parasitological examination The stool samples were examined macroscopically, followed by mi-croscopic examination by native, lugol, and flotation methods.	Infection prevalence rate Cases (47%) vs controls (55.9%) In DM groups, *A. lumbricoides*: 57.5%,, T.trichura: 14.2% *E. histolytica*: 13.3% *G. intestinalis*: 11.7% *T. saginata* or *H.nana*: 3.3%

NGT: normal glucose tolerant; IGT: impaired glucose tolerant; NDDM: newly diagnosed type-2 DM subjects; KDM: known type-2 DM subjects

Table 2: The relationship of helminths and DM with animal models in research

Helminth species	Outcome on DM	References	Animal model	References
Wuchereria bancrofti *Brugia malayi* (lymphatic filariasis)	Protective effect on type 1 DM	Aravindhan et al (2010)	Non-obese diabetic mice infected with *S. mansoni* soluble egg antigen	Hubner et al (2009)
Wuchereria bancrofti *Brugia malayi* (lymphatic filariasis)	Protective effect on type 2 DM	Aravindhan et al (2010)	Non-obese diabetic mice infected with *S. mansoni* soluble egg antigen	Hubner et al (2009)
Enterobius vermicularis (enterobiasis)	No association or not reduced risk for type 1 DM	Bager et al (2012)	Non-obese diabetic mice infected with *S. mansoni* soluble egg antigen	Liu et al (2009)
Schistosoma (schistosomiasis)	Protective effect on type 2 DM	Chen et al. (2012)	Non-obese diabetic mice infected with *S. mansoni* soluble egg antigen	Zaccone et al (2003)
Schistosoma mansoni (schistosomiasis)	Lower islet cell antibody level and lower insulin impairment in type 1 DM	Soliman et al (1996)	NA	NA
Strongyloides stercoralis (strongyloidiasis)	Positive association with type 2 DM	Mendonca et al (2006)	NA	NA
Strongyloides stercoralis (strongyloidiasis)	Reduced risk for type 2 DM	Hays et al (2015)	Non-obese diabetic mice infected with *S. mansoni* soluble egg antigen	Cooke et al (1999)

Chapter 6

Association between helminth infections and diabetes mellitus in adults from the Lao People's Democratic Republic: a cross-sectional study

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6.1 Abstract

Background: As a result of epidemiological transition, the health systems of low- and middle-income countries are increasingly faced with a dual disease burden of infectious diseases and emerging non-communicable diseases. Little is known about the mutual influence of these two disease groups. The aim of this study was to investigate the co-occurrence of helminth infections and diabetes mellitus in adults in Lao People's Democratic Republic (Lao PDR).

Methods: We conducted a cross-sectional study among 1600 randomly selected adults aged 35 and older from four different socio-economical and ecological provinces. Information on socio-demographics, risk factors and health conditions was obtained from personal interviews. Clinical assessments including anthropometry (height, weight, waist and hip circumference) and blood pressure measurements were also conducted. Diabetes was classified based on self-reported diagnoses and a point-of-care glycated haemoglobin (HbA1c) test from finger prick blood samples. Stool samples for helminth diagnosis were examined with formalin-ether concentration technique for intestinal parasitic infections. The independent associations of helminth infections with diabetic status and HbA1c were assessed using multiple regression analyses.

Results: The prevalence of pre-diabetes and diabetes was 37.3% and 22.8%, respectively. Fifty-six percent of diabetic cases were undiagnosed and 85% of diagnosed diabetic cases had poor glycemic control. Participants from rural areas and from southern parts of the country had higher infection rates, with *Opisthorchis viverrini*, being the most common helminth infection (30.5%). We found a positive association between *Taenia* spp. infections and HbA1c (β = 0.117; 95% *CI*: 0.042–0.200) and diabetes mellitus risk (*OR* = 2.98; 95% *CI*: 1.10–8.05). No other helminth species was associated with glycated hemoglobin.

Conclusions: Hyperglycaemia and diabetic rates in Lao PDR are alarmingly high, but consistent with other high rates in the region. Given the high rates of under-diagnosis and poorly-controlled glycaemia in diabetes mellitus patients, routine diabetes screening and treatment is essential for the local healthcare system. Large longitudinal cohorts integrating biomarkers are warranted in the search of causal diabetes mellitus risk factors in the region. Common intestinal helminth infections, including *O. viverrini,* are unlikely to explain the high diabetes mellitus rates observed.

Keywords: Dual burden of disease, *Opisthorchis viverrini*, *Taenia,* Diabetes mellitus, Cross-sectional, Epidemiology, Adults, Lao PDR

6.2 Background

Most low- and middle- income countries (LMICs) are faced with challenges arising from demographic aging and lifestyle changes along with economic development, resulting in an increasing prevalence of non-communicable diseases (NCDs). An estimated 415 million people are currently living with diabetes mellitus (DM) worldwide, and the number is estimated to increase to 642 million in 2040 (1). Similarly, the infectious disease (ID) burden in these countries also remains high. Approximately, 24% of the world's population (more than 1.5 billion people) is infected with one or more species of helminth infections (2). The population of the Lao People's Democratic Republic (Lao PDR) is affected by even higher rates of helminth infections, as a result of high rates of soil-transmitted helminths (STH) such as hookworm (87%), *Trichuris trichiura* (33%)*, Ascaris lumbricoides* (3%) and *Strongyloides stercoralis* (45%)(3-6). Foodborne trematode infections are endemic in all provinces in Lao PDR, however rates are particularly high in the southern provinces and frequently reach 50% and above (7). In a study in the province of Saravane, the prevalence of *Taenia* spp. at the village level reached a prevalence of 12% (8). According to the World Health Organisation (WHO) Lao PDR country profile, 2016, the prevalence of DM in the total population is estimated at 5.6%, and is expected to increase considerably in the near future (9).

Recent epidemiological discoveries have pointed to a potential role of helminth infections in the aetiology of diabetes. Many helminths have evolved to live in human organisms for long periods of time. The strategy against being expelled involves a cross-talk with human innate and adaptive immune responses, which may be mediated in part by alterations in the gut microbiome. The inverse relationship between helminth infections and risk of metabolic disorders has been named "metabolic hygiene hypothesis", after the framework of the hygiene hypothesis for the association between early childhood infections and allergies. Furthermore, inflammation is an established etiological factor for insulin resistance, a precursor phenotype on the pathway to DM (10); however, the metabolic hygiene hypothesis has not been commonly tested. The objective of this study was therefore, to evaluate the epidemiological association of specific helminthic infections with glycaemia and DM in adults from the Lao PDR.

6.3 Methods

Study setting and sample size

A total of 1600 adults aged 35 years and older were recruited in four areas of Lao PDR, each consisting of urban as well as rural sites from the provinces of Vientiane (the

Vientiane capital and Pakgneum district), Luang Prabang (LP city and Namback district), Saravane (Saravane city and Saravane district), and Champasack (Pakse city and Kong district), between March and April, 2016. Study subjects were randomly recruited among inhabitants if they had been living in the selected study areas for the last five years. We assumed a 20% parasitic infection rate in the study area. With 186 enrolled individuals the infection rate could be assessed with a 95% confidence interval (*CI*). We compensated for an expected non-optimal compliance by adding 10% to the sample calculated size. Therefore, 200 persons were enrolled in each of the 4 study areas. Data collection took place in March–April 2016. All 1600 participants answered a questionnaire, had a clinical examination and blood taken through finger prick. They also provided two stool samples each. The data collectors were first year master students from the Francophone Institute for Tropical Medicine (IFMT), Vientiane, Lao PDR (today named International Program for Health in the Tropics, now integrated in the Lao Tropical and Public Institute [Lao TPHI] in Vientiane) who had undergone a rigorous training for each segment of data collection.

Questionnaire and clinical information

Each participant was assigned a specific six-digit identification number, reflecting the study site, the village and the household number. A semi-structured questionnaire was developed to collect information on socio-demographic factors, risk factors for chronic diseases, previous and current health problems, as well as knowledge and perception of diseases of interest for the study. The questionnaire was translated from English to the local language for the interviews.

The clinical examination included a general assessment of the participant's health status, including the measurement of arterial blood pressure, height, weight, waist and hip circumference, as well as haemoglobin and glycated haemoglobin (HbA1c) in capillary blood.

Blood pressure was measured three times over an interval of five minutes between each reading using the Omron M6 AC (Hoofdoorp, Netherlands) upper arm digital blood pressure monitor. Participants were seated for 15 minutes before the blood pressure assessment and the mid-arm circumference was also measured with the use of an appropriate semi-rigid preformed arm cuff. The mean systolic and diastolic blood pressure readings were noted.

Height was measured using a SECA 206 roll-up measuring tape with wall attachment. (SECA GmbH & Co. KG, Hamburg, Germany) The height was recorded in centimetres with one decimal.

Weight was measured using the non-automatic personal scale, SECA 877 (SECA GmbH & Co. KG, Hamburg, Germany). Participants were asked to take off shoes, coat and heavy clothes before being weighed. The weight was recorded with one decimal.

Waist and hip circumference was measured using the SECA 203 measuring tape (SECA GmbH & Co. KG, Hamburg, Germany). The waist circumference measured was the area between the ribs and iliac crest, and the hip circumference was the maximum circumference between the iliac crest and the crotch. Measurements were recorded in centimetres with one decimal.

Haemoglobin level was measured using the point-of-care anaemia screening, HemoCue Hb 301 System (HemoCue AB, Ängelholm, Sweden). Results were reported to the nearest 0.1 g/L.

Glycated haemoglobin (HbA1c)/DM status assessment: HbA1c values reflect plasma glucose concentration over an eight to 12 weeks period before the measurement. It is a convenient screening test for diabetes as there is no fasting requirement. HbA1c concentration in blood obtained by a finger-prick test was measured using the point-of-care instrument Afinion AS 100 analyser and cartridges (Alere Inc. Waltham, Massachusetts, USA). The cut-offs of the American Diabetes Association (11) were applied: normal: <5.7; pre-DM: 5.7–6.4; and DM: ≥6.5. Subjects which self-reported a diagnosis and treatment of DM were classified as DM, irrespective of their HbA1c blood concentration. DM categories were further subdivided into diagnosed and treated DM; diagnosed DM, but untreated DM and undiagnosed DM. Newly diagnosed DM cases with HbA1c levels ≥6.5% were referred to a health clinic or hospital for confirmation of DM.

Parasitological examination

Two faecal samples were collected consecutively for two days for each participant and fixed in 10% formalin. The fixed samples were sent to the microbiology laboratory at Khon Kaen University, Khon Kaen, Thailand, in which, the formalin ether concentration technique (FECT) was used for helminth diagnosis.

Data management and analysis

Data was double-entered and subjected to validation checks using EpiData 3.1 (EpiData Association, Odense, Denmark). A complete case analysis (individuals with stool samples, HbA1c results, and complete covariate information) was conducted to investigate the association of explanatory variables (helminths; covariates) with the outcome variable (HbA1c; DM) using STATA 14.1 (StataCorp; College Station, TX, USA). Descriptive

statistics of the participants characteristics were reported as frequencies and percentages for categorical variables and means and standard deviations (SD) for continuous variables. The sex-stratified DM categories according to the measured HbA1c concentrations were cross-tabulated with self-reported DM diagnosis and treatment. The helminth infection prevalence was stratified by study sites as well as the type of study area (rural vs urban). The distribution of covariates and helminth infections was presented separately for non-DM (non self-reported DM; normal HbA1c), pre-DM (non self-reported DM; pre-DM HbA1c), and DM groups (self-reported DM diagnosis or diabetic HbA1c). The socio-economic status (SES) of participants was estimated using a household-based asset approach. SES scores were constructed using principle component analysis (PCA). Participants were categorized into three groups; poorest, second poorest and least poorest (12). Chi-square (χ^2) tests and analysis of variance tests were conducted to investigate the unadjusted association between covariates and DM categories. Linear (outcome HbA1c measurements) and logistic (outcome DM status) regression models with random intercepts of study sites were performed to explore the independent relationship between helminth infections and HbA1c measurements and diabetes, respectively. Participants self-reporting a physician diagnosis of DM and medical DM treatment (N = 54) were excluded from these regression analyses. The regression models were adjusted for a set of preselected potential confounders (study sites, age, sex, educational level, SES status, alcohol consumption, smoking status, haemoglobin levels, body mass index [BMI] and physical inactivity). A two-sided P value <0.05 was considered as a statistically significant level.

6.4 Results

Among the 1604 participants, 1528 (95.3%) were considered in the analysis. The observed prevalence of pre-diabetes and diabetes was 37.3% and 22.8%, respectively (Fig 1). Table 1 summarizes the total and sex-stratified distribution of DM and pre-DM according to HbA1c levels, both, for the entire study sample and stratified by self-reported DM diagnosis and treatment. Among the total sample, 614 participants had HbA1c values in the normal range, in which, five self-reported DM; 585 in the pre-DM range, in which, 15 self-reported DM; and 329 in the DM range, in which, 135 self-reported DM. The distribution of DM categories did not differ considerably between males and females. Fifty-six percent (n = 194) participants from a total of 349 participants with DM were unaware of their disease (Fig 1). Among the 155 participants self-reporting a DM diagnosis, only 39% (n = 61) reported some kind of treatment, while only 35% (n = 54) reported intake of physician-prescribed anti-

diabetic treatment. In more than 85% of patients with self-reported DM, irrespective of undergoing treatment or not, HbA1c concentrations measured were still in the diabetic range.

Table 1: Diabetes status according to HbA1c measurements, stratified by sex, self-reported diabetes diagnosis and treatment status

DM category according to HbA1c level[1]	**Total** *N* **(%)**	**Male** *N* **(%)**	**Female** *N* **(%)**
All study participants (*N* = 1528)			
Normal	614 (40.2)	183 (40.9)	431 (39.9)
Pre-DM	585 (38.3)	165 (36.8)	420 (38.9)
DM	329 (21.5)	100 (22.3)	229 (21.2)
Mean (Standard Deviation)	6.4 (1.9)	6.4 (1.9)	6.4 (1.9)
Self-reported DM cases (*N* = 155)			
Normal	5 (3.2)	3 (6.1)	2 (1.9)
Pre-DM	15 (9.7)	3 (6.1)	12 (11.3)
DM	135 (87.1)	43 (87.7)	92 (86.8)
Self-reported DM cases with any treatment (*N* = 61)			
Pre-DM	8 (13.1)	0 (0)	8 (17.0)
DM	53 (86.9)	14 (100)	39 (83.0)
Self-reported DM cases with a physician diagnosis and currently taking DM drugs (*N* = 54)			
Pre-DM	7 (13.2)	0 (0)	7 (17.1)
DM	46 (86.8)	12 (100)	34 (82.9)

[1] According to cut-offs of the American Diabetes Association [11]: Normal: <5.7; Pre-DM: 5.7–6.4; DM: ≥6.5

DM: Diabetes mellitus; HbA1c: Glycated haemoglobin

Table 2 shows the prevalence of helminth infections stratified by rural and urban areas as well as by the four study provinces. Helminth infections were more frequent in rural areas compared to urban areas and in the two southern provinces compared to the two Northern provinces. The two southern provinces in Lao PDR and along the Mekong River (Saravane and Champasack) had overall infection rates of 49% and 44.9%, respectively, mostly due to *O. viverrini* (43% and 40%, respectively). In fact, *O. viverrini* was the most frequent trematode followed by minute intestinal flukes and *Paragonimus* spp. with prevalence rates of

30.5%, 6.3% and 0.3 %, respectively. The overall nematodes infection rates were 7.7% including hookworm (4.8%), *S. stercoralis* (2.6%), *T. trichiura* (0.5%) and *A. lumbricoides* (0.3%), respectively. We also found an infection rate of 2.2% for *Taenia* spp.

Table 2: Prevalence (*N*, prevalence) of helminth infections in the study area

Helminth infections	Total (*N* = 1528)	Rural (*n* = 766)	Urban (*n* = 762)	Northern Province Vientiane[1] (*n* = 378)	Northern Province (*n* = 363)	Southern Province Saravane[1] (*n* = 396)	Southern Province Champasack[1] (*n* = 391)
Any infection	539 (35.3)	332 (43.3)	207 (27.2)	113 (29.9)	58 (16.0)	193 (48.7)	175 (44.8)
Any trematode infection	473 (31.0)	286 (37.3)	187 (24.5)	108 (28.6)	34 (9.4)	174 (43.9)	157 (40.2)
Opisthorchis viverrini	466 (30.5)	282 (36.8)	184 (24.1)	106 (28.0)	33 (9.1)	171 (43.2)	156 (39.9)
Minute intestinal flukes	96 (6.3)	71 (9.3)	25 (3.3)	8 (2.1)	0 (0)	45 (11.4)	43 (11.0)
Paragonimus spp.	4 (0.3)	2 (0.3)	2 (0.3)	1 (0.3)	1 (0.3)	1 (0.3)	1 (0.3)
Any nematode infection	117 (7.7)	87 (11.4)	30 (3.9)	5 (1.3)	26 (7.2)	52 (13.1)	34 (8.7)
Hookworm	73 (4.8)	55 (7.2)	18 (2.4)	2 (0.5)	10 (2.8)	44 (11.1)	17 (4.4)
Strongyloides stercoralis	40 (2.6)	28 (3.7)	12 (1.6)	2 (0.5)	12 (3.3)	9 (2.3)	17 (4.3)
Ascaris lumbricoides	4 (0.3)	4 (0.5)	0 (0.0)	1 (0.3)	3 (0.8)	0 (0)	0 (0)
Trichuris trichiura	7 (0.5)	5 (0.7)	2 (0.3)	0 (0.0)	4 (1.1)	2 (0.5)	1 (0.3)
Cestodes (*Taenia* spp.)	34 (2.2)	25 (3.3)	9 (1.2)	12 (3.2)	3 (0.8)	13 (3.3)	6 (1.5)

[1] in each province participants from urban and rural sites were recruited

Table 3 compares the characteristic of participants and helminth infection rates across DM status categories, defined by the combination of self-reported DM and HbA1c concentration. The mean age of participants was 54.9 years (SD: 12.0 years, range: 35–95 years), 70% were women, 50% lived in rural settings, 94% belonged to the Lao ethnic group, 16% were illiterate and 31% belonged to the poorest category. With regard to lifestyle factors, 32% reported to be ever smokers, 48% to be ever alcohol drinkers and 19% to be physically inactive. The mean waist circumference was 82.8 cm in men and 80.7 cm in women (recommended cut-off points for Asians: 90 cm in men and 80 cm in women)(13). The mean Hb concentration was 131.3 mg/dl in men and 119.1 mg/dl in women (WHO cut-off points for non-anaemic Hb values are ≥130 g/L (male); ≥110 g/L (female)). As expected,

participants with DM were more likely to be older, to be of higher socio-economic status and to score higher on the anthropometric parameters including BMI, waist circumference, hip circumference and waist-hip ratio. Participants without diabetes exhibited the highest overall infection, with trematode and *O. viverrini* among this unadjusted comparison, whereas there was a tendency for *Taenia* spp. to be more common in subjects with DM.

Table 3: Participants characteristics according to DM categories (*N* = 1528)

Covariates	Total (*N*, %)	Categories of DM (N, %)[1]			*P-value
		No DM (*n* = 609)	Pre-DM (*n* = 570)	DM (*n* =349)	
Age					**<0.001**
35–49 years	535(35.0)	303(49.7)	163(28.6)	69(19.8)	
50–60 years	534(34.9)	177(29.1)	199(34.9)	158(45.3)	
61–95 years	459(30.1)	129(21.2)	208(36.5)	122(34.9)	
Mean, SD	54.9(12.0)				
Female (%)	1080(70.7)	429(70.4)	408(71.6)	243(69.6)	0.83
Rural (%)	766(50.1)	322(52.9)	276(48.4)	168(48.1)	0.12
Ethnicity (Lao)	1438(94.1)	564(92.6)	540(94.7)	334(95.7)	0.59
Education					0.05
Illiterate	247(16.2)	80(13.1)	105(18.4)	62(17.8)	
Primary level	719(47.0)	286(47.0)	270(47.4)	163(46.7)	
Secondary level	562(36.8)	243(39.9)	195(34.2)	124(35.5)	
Socio-economic status					**0.002**
Poorest	505(33.1)	219(36.0)	199(34.9)	87(24.9)	
Second least poorest	514(33.6)	208(34.1)	186(32.6)	120(34.4)	
Least poorest	509(33.3)	182(29.9)	185(32.5)	142(40.7)	
Ever smokers	491(32.1)	196(32.2)	177(31.1)	118(33.8)	0.64
Ever alcohol drinkers	733(48.0)	354(58.1)	244(42.8)	135(38.7)	**<0.001**
No physical activity	288(18.8)	112(18.4)	122(21.4)	54(15.5)	0.13
Weight (kg; mean, SD)	58.1(11.9)	55.6(11.0)	58.3(11.8)	61.9(12.5)	**<0.001**
Height (cm; mean, SD)	153.6(7.2)	153.8(7.1)	153.0(7.1)	154.0(7.4)	0.08
BMI (kg/m^2; mean, SD)	24.6(4.5)	23.5(4.1)	24.8(4.5)	26.0(4.5)	**<0.001**
Hip circumference (cm;	93.2(10.0)	91.0(9.7)	94.1(10.0)	95.8(9.7)	**<0.001**

mean, SD)					
Waist circumference (cm; mean, SD)	81.2(11.8)	77.4(10.8)	82.3(11.7)	86.5(11.2)	0.12
Waist-hip ratio (mean, SD)	0.87(0.07)	0.85(0.06)	0.87(0.06)	0.90(0.06)	**<0.001**
Hb concentration (mg/dl; mean, SD)	122.7(17.8)	122(18)	123(18)	125(18)	**0.04**
Helminth infections					
Any infection	513(33.6)	224(36.8)	189(33.2)	100(28.7)	**0.04**
Any trematode infection	473(31.0)	211(34.6)	175(30.7)	87(24.9)	**0.03**
Opisthorchis viverrini	466(30.5)	207(34.0)	169(29.6)	90(25.8)	**0.03**
Minute intestinal flukes	96(6.3)	37(6.1)	41(7.2)	18(5.2)	0.62
Paragonimus spp.	4(0.3)	1(0.2)	2(0.3)	1(0.3)	0.82
Any nematode infection	117(7.7)	51(8.4)	49(8.4)	17(4.9)	0.16
Hookworm	73(4.8)	34(5.6)	25(4.4)	14(4.0)	0.23
Strongyloides stercoralis	40(2.6)	13(2.1)	21(3.7)	6(1.7)	0.15
Ascaris lumbricoides	4(0.3)	2(0.3)	2(0.3)	0(0.0)	0.56
Trichuris trichiura	7(0.5)	4(0.7)	3(0.5)	0(0.0)	0.34
Cestodes/*Taenia* spp.	34(2.2)	10(1.6)	11(1.9)	13(3.7)	0.08

* *P*-value comparing the distribution of the respective factor between DM categories
[1] categorization based on a positive self-report of DM diagnosis and otherwise based on the HbA1c concentrations
DM: Diabetes mellitus

Table 4 shows the independent associations of infection groups and single infections with HbA1c in the study population after exclusion of subjects with physician-diagnosed and treated DM. Results are presented with and without adjustment for BMI and physical inactivity, which may in part be mediators of the association between helminth infections and HbA1c. Positive associations between *Taenia* spp. infections and HbA1c were observed, in both, models containing infection groups of single infections and models with and without adjustment for BMI and physical activity (largest effect estimate in model of infections groups and adjusting for BMI and physical activity: $\beta = 0.117$; 95% *CI*: 0.042–0.200). Interestingly, this positive association seems to be driven by an association with HbA1c in the

diabetic range. In healthy subjects without pre-DM or DM, we found an inverse relationship between *Taenia* spp. and HbA1c measurements (β = -0.049; 95% *CI*: -0.075–0.022) (Additional file 1). No association of HbA1c with any other infection or infection group was observed.

Table 4: Independent association of single infections and infection groups with HbA1c in all participants

Infections	Adjusted for other infections, study site, age, sex, SES status, education status, smoking status, alcohol consumption and haemoglobin level		Additionally adjusted for BMI and physical inactivity	
	β	**95% *CI***	**β**	**95% *CI***
Grouped infections[2] (*N* = 1474)				
Nematodes	-0.040	-0.081–0.002	-0.035	-0.076–0.006
Trematodes	-0.003	-0.027–0.021	-0.0003	-0.023–0.024
Cestodes *Taenia* spp.	**0.112**	**0.037–0.188**	**0.117**	**0.042–0.200**
Single infection[3] (*N* = 1466) [4]				
Hookworm	-0.030	-0.083–0.021	-0.025	-0.076–0.027
Opisthorchis viverrini	-0.009	-0.035–0.017	-0.002	-0.025–0.022
Minute intestinal flukes	0.021	-0.028–0.069	0.011	-0.034–0.056
Strongyloides stercoralis	-0.043	-0.128–0.014	-0.046	-0.116–0.024
Trichuris trichiura	-0.043	-0.202–0.115	-0.063	-0.220–0.094
Taenia spp.	**0.114**	**0.039–0.190**	**0.116**	**0.042–0.192**

[1]Participants excluding participants self-reporting a physician-diagnosis of DM and intake of DM medication

[2]Model including infection groups nematodes (yes vs no), trematode infection (yes vs. no), and cestodes (yes vs no)

[3]Model including single infections hookworm (yes vs no), *O. viverrini* (yes vs no), minute intestinal flukes (yes vs no), *Strongyloides stercoralis* (yes vs no), *Trichuris trichiura* (yes vs no) and *Taenia* spp./cestodes (yes vs no)

[4]Subjects with other rare types of infections were excluded from this analysis.

CI: Confidence interval

Table 5 shows the independent associations of single helminth infections with DM compared to non-DM after excluding subjects with a self-reported physician diagnosis of DM and intake of DM medication, and leaving the pre-DM category out. Results are again presented with and without adjustment for BMI and physical inactivity. Consistent with our findings on the infection-HbA1c associations, we found that having a Cestode infection, but not any other infections was associated with an increased risk of DM (Adjusted *OR* = 2.98, 95% *CI*: 1.10–8.05). The independent association of other factors with DM risk in the expected directions validate the assessment of DM and HbA1c: age (*OR* = 1.05, 95% *CI*: 1.04–1.08), being male (*OR* = 1.42, 95% *CI*: 0.89–2.00), highest socio-economic status (*OR* = 1.70, 95% *CI*: 1.08–2.67), ever smoker (*OR* = 1.89, 95% *CI*: 1.22–2.92), and exhibiting high Hb concentration (*OR* = 1.02, 95% *CI*: 1.15–1.25), and high BMI (*OR* = 1.20, 95% *CI*: 1.15–1.25). Interestingly, self-reported alcohol consumption (*OR* = 0.54, 95% *CI*: 0.38–0.76) was inversely associated with DM risk. These results did not substantially change when pre-DM and DM were jointly compared to the pre-DM or DM group (Additional file 2).

Table 5: Independent association of single infections with DM compared to non-DM, with and without adjustment for BMI and physical inactivity in all participants (*N* = 892[1])

DM status compared to Normal[2]	*OR*[3]	95% *CI*	*P*-value	*OR*[4]	95% *CI*	*P*-value
Hookworm	0.79	0.36–1.72	0.56	0.94	0.42-2.10	0.88
Opisthorchis viverrini	0.76	0.52–1.10	0.15	0.87	0.58–1.28	0.46
Minute intestinal flukes	1.38	0.68–2.78	0.37	1.20	0.57–2.52	0.63
Strongyloides stercoralis	0.65	0.19–2.30	0.51	0.65	0.15–2.72	0.55
Trichuris trichiura	1.00	Omitted		1.00	Omitted	
Taenia spp.	2.59	0.98–6.87	0.06	**2.98**	**1.10–8.05**	**0.03**
Study sites(Vientiane as reference)						
Lung Prabang	0.75	0.47–1.20	0.24	0.80	0.48–1.31	0.37
Saravane	0.74	0.47–1.28	0.32	0.89	0.52–1.51	0.66
Champasack	0.76	0.48–1.20	0.24	0.85	0.52–1.39	0.53
Socio-economic status(Poorest as reference)						
Second least poorest	**1.51**	**1.00–2.27**	**0.04**	1.30	0.84–1.99	0.24
Least poorest	2.16	1.42–3.31	**<0.001**	**1.70**	**1.08–2.67**	**0.02**
Age	1.05	1.04–1.07	**<0.001**	**1.05**	**1.04–1.08**	**<0.001**
Gender (Male)	1.64	1.05–2.56	**0.03**	1.42	0.89–2.27	0.14
Education(Illiterate as reference)						
Primary	0.92	0.57–1.47	0.72	0.71	0.43–1.18	0.19
Secondary	0.85	0.49–1.45	0.55	0.69	0.39–1.21	0.20
Ever smokers	1.53	1.01–2.32	**0.04**	**1.89**	**1.22–2.92**	**0.004**
Ever alcohol drinkers	0.54	0.38–0.76	**<0.001**	**0.48**	**0.33–1.03**	**<0.001**
Hb concentration	1.02	1.01–1.03	**<0.001**	**1.02**	**1.01–1.03**	**0.002**

+ BMI	-	-	-	**1.20**	**1.15–1.25**	**<0.001**
+ Physical activity	-	-	-	1.19	0.76–1.87	0.45

[1] Subjects with pre-DM and participants self-reporting a physician diagnosis of DM and intake of DM medication and with other rare types of infections were excluded from this analysis

[2] Categorization based on a positive self-report of DM diagnosis and otherwise based on the HbA1c concentrations

[3] Mutually adjusted for variables listed

[4] Additionally adjusted for BMI and physical inactivity

CI: Confidence interval; *OR*: Odd ratio; BMI: Body mass index; DM: Diabetes mellitus

6.5 Discussion

We found alarmingly high rates of DM and pre-DM in this adult study population in Lao PDR. Local population-based HbA1c findings for comparison are not available in Lao PDR, however, the prevalence found in this study is consistent and comparable to that of the province of Guangdong, an economically well-developed and urbanized area in Southern China, in which the prevalence is 22% (14). The study in China estimated the diabetes prevalence based on a combination of self-reported DM, HbA1c measured in capillary finger-prick blood as well as venous blood fasting glucose and glucose tolerance testing. This suggests that the observed DM prevalence in Lao PDR, may even be higher than reported here as adding glucose to the DM screening tests results in the identification of additional cases (14, 15). The high pre-DM and DM rates with a tendency for earlier onset in Asian populations remain partly unexplained (16). According to the results of this current study common helminth infections in Lao PDR may not explain the high DM and pre-DM prevalences.

The current findings on the prevalence and distribution of helminth infections are consistent with previous studies in Lao PDR. Multiple helminth infections of different trematode, nematodes and cestode species were also common as documented in previous literature (17-20). The high prevalence rates of the trematode infection, particularly with *O. viverrini,* is consistent with previous studies (21). Minute intestinal flukes and hookworm were also very frequently diagnosed. These helminths are generally more common in Lao PDR and the Mekong sub-region (19, 22). In our study we found 2.2% participants infected with *Taenia* spp.*,* which is in general a rather high rate but comparable to previous observations from our team (23). The national deworming program from the Ministry of Health implements biannual treatment of soil-transmitted helminths in school-children in collaboration with the ministry of education. In recent years, several rounds of mass-treatment were conducted with praziquantel in selected districts of Southern Lao PDR. While these control measures have had a considerable impact on the infection rates of soil-transmitted helminths, the prevalence of liver fluke infections were not reduced.

We have previously reported highly prevalent liver pathologies in rural Lao PDR, as documented by an ultrasound-based study in Saravane province, one of the current study areas (24). We associated severe liver morbidity with *O. viverrini* (21), known to be a main risk factor for cholangiocarcinoma (25, 26). The absence of an association between *O. viverrini* infection and DM risk, suggests that *O. viverrini* related liver pathology may not contribute to DM development and the high rate of hyperglycaemia. In contrast, fatty liver disease,

especially non-alcoholic fatty liver disease, is viewed as hepatic manifestation of the metabolic syndrome, is associated with insulin resistance, and was previously found to be an independent predictor of incident type 2 DM (27), due to chronic inflammation of the liver or hepatokine secretion. Fatty liver disease and *O. viverrini* associated liver pathology are different entities, which can in principle be differentiated by ultrasound, but the histological examination of liver biopsies remains the diagnostic gold standard. The independent and combined effects of *O. viverrini* infections, associated liver pathologies and fatty liver disease on incident DM need further investigation as the interactions are likely complex and possible in opposite directions. Future studies should also consider *O. viverrini* related modifications of the gut microbiome given the strong evidence for its relation to type 2 DM (28, 29).

Both HbA1c and DM status were positively associated with *Taenia* spp. infection. Taeniasis is an intestinal parasitic infection and is acquired by the consumption of raw or undercooked meat (pork or beef) (30). It is common in developing countries of Latin America, Asia, and Africa, and associated with poor hygienic and sanitary conditions (inadequate use of latrines or open-air defecation, traditional pig farming, lack of regulation on meat inspection and inadequate water supply) (30). *Taenia* spp. infection is listed as one of the 17 neglected zoonotic diseases by WHO, which can be preventable and treatable. Individuals with intestinal *Taenia* infection are usually asymptomatic. *T. solium* is the cause of cysticercosis if parasite eggs are faecal-orally acquired. Cysticercosis may lead to a severe disease, particularly if the central neural system (neuro-cysticercosis) is affected (31). There was no diagnosis of cysticercosisin our study sample. . All taeniasis patients in our study had a history of raw meat consumption (92% beef and 32% pork). People infected came mostly from rural areas of Vientiane and Saravane provinces. Co-infections with other helminths were also detected in half of *Taenia* sp. infected individuals, mostly with *O. viverrini* and/or minute intestinal flukes, hookworm and *S. stercoralis*. One study examining the effect of immune modulation induced by *Taenia crassiceps* infection on the outcome of multiple low dose of Streptozotocin-induced diabetes (MLDS) reported that *T. crassiceps* infection might protect against MLDS, irrespective of the host's genetic background. To the best of our knowledge, this is the first study to report an association between *Taenia* sp. infection and DM as well as HbA1c.

A limited number of studies have started to explore the interrelation between helminth infections and diabetes. Endemic helminth infections are thought and in part shown to affect insulin sensitivity and resistance through immune-modulating properties and by reducing

energy intake and altering energy balance (32). Yet, epidemiological evidence remains poor, and inconsistent, and points to infection-specific associations with DM. Some previous studies have reported a negative association between soil-transmitted helminth infections and insulin resistance (33), between filarial infection and type 1 and type 2 DM (34), and between *Schistosoma japonicum* infections and type 2 DM (35). An infection with *S. stercoralis* was found positively associated with severe DM (36). We reported a positive association of *H. pylori* infections with HbA1c in school children from poor neighbourhoods in South Africa, but neither a cross-sectional association with other common helminth infections nor a change in HbA1c as a result of anti-helminthic treatment (37). It has been shown that socioeconomic, environmental and behavioural factors influence the prevalence and intensity of helminth infections and could therefore, in part, be confounders as well as mediators of any association with diabetes (18, 38). It is thus, important to consider the recently published results from the first randomized placebo-controlled SUGARSPIN trial, investigating the effect of anthelmintic treatment on whole-body insulin sensitivity in a large Indonesian population sample (39). Albendazole treatment had no effect among participants without any or only a single species helminth infection measured (hookworm [*Ancylostoma duodenale*, *Necator americanus*]; *A. lumbricoides; T. trichiura*; *Strongyloides stercoralis*), but resulted in high homeostatic model of insulin resistance, a measure of insulin resistance, in the presence of multiple species infection at baseline. No effect on HbA1c was observed, though.

Our study has a number of strengths. First, this is the only study to date assessing DM prevalence in Lao PDR, based on Hba1c measurements. Participants from the rural and urban area of the provinces located in the middle and southern part of the country were included; therefore, the findings reflect the prevalence of helminths and DM status of different populations from different geographical settings. Access to curative health services is very low in rural settings. Therefore, our results underline the needs for peripheral curative health services for DM diagnosis and management. Second, no previous study investigated the dual burden of diseases of DM and helminth infections in adults in Lao PDR. In fact, this is the first time the association of *O. viverrini* infection with DM was investigated. Third, we used an internationally certified HbA1c test, which was able to capture most of the previously diagnosed DM cases. The HbA1c point-of-care method applied was validated for use in hot and remote low-income settings in the context of our previous study in South Africa (37). The validity of our HbA1c findings is further supported by their positive association with validated DM risk factors such as age, BMI and smoking. Finally, to achieve a satisfactory

sensitivity for the helminth diagnosis, we examined two stool samples per person, which is known to increase the sensitivity (40).

There are however, some limitations in our study. It is known that *T. solium*, *T. saginata* and *T. asiatica* are endemic in Asia (41). In our study, we did not distinguish the *Taenia* species, and hence could not study the species specific associations with HbA1c in the blood. Moreover, due to the cross-sectional nature of our study, we were not able to identify the time-course relationship between infections and DM development. Reverse causation is a concern as DM patients have an increased susceptibility to infections due to their immune dysfunction (42). The observed association between *Taenia* spp. and HbA1c could therefore be real, a chance finding, explained by confounding or due to reverse causation. Finally, we could not rule out other associated infectious or non-infectious conditions of study participants, which might influence the blood glucose levels and confound, modify or mediate the observed infection-DM associations. Multi-parasitism is very common in different provinces in Lao PDR and a significant association has been observed between *S. mekongi* and hookworm in Southern Lao PDR (23), as well as between *S. mansoni* and hookworm in Côte d'Ivoire (43). Since each parasite has a different effect on blood sugar, energy balance, and immunity, co-infections status may play an important role in studying dual disease burden.

6.6 Conclusions

Our study found an alarmingly high level of uncontrolled hyperglycaemia in both, urban and rural Lao PDR. These results are not consistent, with a strong role of common infections, such as *O. viverrini,* explaining the high diabetes burden observed. Larger and longitudinal studies including biomarkers and liver ultrasounds are warranted to further study the causes of DM in Lao PDR. The Lao PDR health system must work on strengthening its healthcare services in the domain of DM screening and treatment.

List of abbreviations

BMI: Body mass index, *CI*: Confidence interval, DM: Diabetes mellitus, FECT: Formalin-ether concentration technique, ID: Infectious diseases, IFMT: The Francophone Institute for Tropical Medicine, OR: Odd ratio, Lao PDR: Lao People's Democratic Republic, LMICs: Low- and middle income countries, NCDs: Non-communicable diseases, SD: Standard deviation, STH: Soil-transmitted helminths, WHO: World Health Organization

Declarations

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Ethics approval and consent to participate

The National Ethics Committee for Health Research (NECHR) of the Ministry of Health (MoH) of Lao PDR (approval No. 017 NIOPH/NECHR, 14 March 2016) approved the study protocol. After research aim, methods, risks and benefits of the study were explained in detail to participants, district and provincial health authorities, written informed consent was also obtained from each participant prior data collection. Diagnosed helminth infection was treated according to the national treatment guidelines.

Consent for publication

Not Applicable

Availability of data and materials

The data from this study are available on request from corresponding author.

Competing interests

We declare that we have no conflict of interest.

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Authors' contributions

NSNH, PO, PP, DR and NPH designed the study. PP, SS, MV, VPN, XDT, PSY, NAH, NDS, AH, BS, DX, DRR, PB, PPP, SDS, YXL,KT, XSX, PTY developed the study protocol, performed the field work and contributed to the data analysis. BS conducted the stool analysis. NSNH, PO, PP, DR and NPH performed the final analysis. NSNH and NPH wrote the first manuscript version. PO, PP, ICE, MI, DR and NPH revised the manuscript. All authors read and approved the final version of the manuscript.

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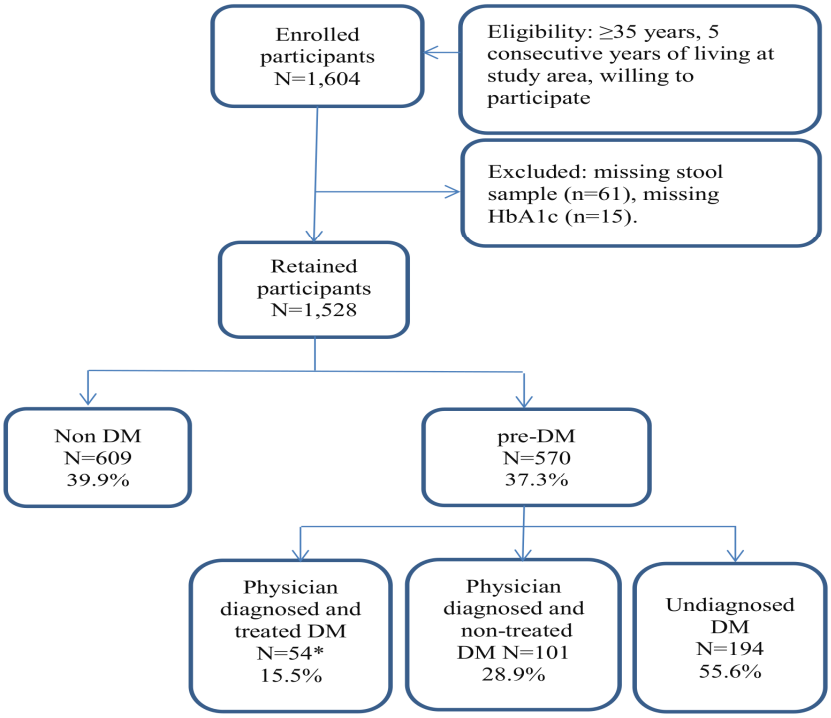

#categorization based on a positive self-report of DM diagnosis and otherwise based on the HbA1c concentrations

*subjects self-reporting a physician diagnosis and taking DM medication were excluded from multivariable regression models on the association between infections and HbA1c or DM

DM: Diabetes mellitus

Fig 1: Flow chart of the study sample and distribution of pre-diabetes and diabetes

Additional files

Additional file 1: Independent association of single infections and infection groups with HbA1c in participants with HbA1c levels in the normal range (columns to the left) (N = 609) and in the normal and prediabetic range (columns to the right) (N = 1179), excluding participants self-reporting a physician-diagnosis of DM.

Additional file 2: Independent association of single infections with pre-DM and DM status combined and compared to non-DM, with and without adjustment for BMI and physical inactivity, excluding participants self-reporting a physician diagnosis of DM and intake of DM medication (N = 1466[1])

Chapter 7

Association between gastrointestinal tract infections and glycated hemoglobin in school children of poor neighborhoods in Port Elizabeth, South Africa

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7.1 Abstract

Background: Low- and middle-income countries are facing a dual disease burden with infectious diseases (e.g., gastrointestinal tract infections) and non-communicable diseases (e.g., diabetes) being common. For instance, chronic parasite infections lead to altered immune regulatory networks, anemia, malnutrition, and diarrhea with an associated shift in the gut microbiome. These can all be pathways of potential relevance for insulin resistance and diabetes. The aim of this study was to investigate the association between common gastrointestinal tract infections and glycemia in children from non-fee paying schools in South Africa.

Methodology: We conducted a cross-sectional survey among 9- to 14-year-old school children in Port Elizabeth. Stool and urine samples were collected to assess infection status with parasitic worms (e.g., *Ascaris lumbricoides*, *Enterobius vermicularis*, and *Trichuris trichiura*), intestinal protozoa (e.g., *Cryptosporidium parvum* and *Giardia intestinalis*), and the bacterium *Helicobacter pylori*. Glycated hemoglobin (HbA1c) was measured in finger prick derived capillary blood. All children at schools with a high prevalence of helminth infections and only infected children at the schools with low infection rates were treated with albendazole. The association of anthelmintic treatment with changes in HbA1c 6 months after the drug intervention was also investigated.

Findings: A high prevalence of 71.8% of prediabetes was measured in this group of children, with only 27.8% having HbA1c in the normal range. *Helicobacter pylori* was the predominant infectious agent and showed an independent positive association with HbA1c in a multivariable regression analysis (β=0.040, 95% confidence interval (CI) 0.006-0.073, $p<0.05$). No association of HbA1c with either any other infectious agent or albendazole administration was found.

Conclusion: The role of *H. pylori* in diabetes needs confirmation in the context of longitudinal treatment interventions. The specific effect of other gastrointestinal tract infections on glycemia remains unclear. Future studies should integrate the measurement of biomarkers, including immunological parameters, to shed light on the potential mediating mechanisms between parasite infections and diabetes.

7.2 Author summary

Parasitic worms (e.g., pinworm, roundworm, and whipworm), intestinal protozoa (e.g., *Cryptosporidium parvum* and *Giardia intestinalis*), and the bacterium *Helicobacter pylori* persist at high rates in the gastrointestinal tract of people from low- and middle-income countries. These infectious agents are increasingly paralleled by high rates of non-communicable diseases (NCDs), such as diabetes. We studied the association of glycemia, measured as HbA1c with common gastrointestinal tract infections among school children aged 9-14 years from disadvantaged neighborhoods in Port Elizabeth, South Africa. Our goal was to deepen the understanding of whether specific gastrointestinal tract infections might be early life determinants of elevated HbA1c levels that might lead to diabetes. We found that the bacterium *H. pylori* was very common among our group of children with a positive association with hyperglycemia. None of the other infectious agents showed such an association. Additional, longitudinal studies are needed to determine whether there is causality for the observed association between *H. pylori* and hyperglycemia. The integration of biomarkers will allow studying mediating mechanisms.

7.3 Introduction

In low- and middle-income countries (LMICs), the dual disease burden stemming from infectious diseases (IDs) and non-communicable diseases (NCDs) poses a challenge to population health and the health systems. Soil-transmitted helminths and schistosomes are estimated to infect over a billion individuals in LMICs [1, 2] and cause abdominal pain, diarrhea, poor cognitive development, malnutrition, and anemia. As a consequence of such symptoms, school and work performance is affected and physical activity levels compromised [3]. Helminthiases are often chronic, a result of both under-treatment and re-infection. Soil-transmitted helminthiasis, schistosomiasis, and intestinal protozoa infection are intimately connected with poverty, partially explained by lack of clean water, sanitation, and hygiene [4].

NCDs are gaining importance, also in LMICs [5]. For example, the frequency of diabetes mellitus (DM) is rising worldwide, and South Africa is among the top five countries in Africa with an estimated DM prevalence of 9.2% [6]. This can be attributed primarily to aging, population growth, increasing rates of unhealthy dietary habits, a sedentary lifestyle, and obesity. While NCDs and DM particularly affect older people, it is generally accepted that early life exposures contribute to the accumulation of molecular damage and a higher disease risk later in adulthood [7].

Little is known about how common parasite infections affect glucose homeostasis and DM etiology, particularly at young age. It is conceivable that parasite infections influence the DM risk through different pathways and in opposite directions [8]. On the one hand, parasite-induced alterations of immune regulatory networks, which have evolved to prolong survival in the human intestines, may stimulate anti-inflammatory pathways and decrease the risk of obesity-induced insulin resistance. Malnutrition, diarrhea and, as a result, low body weight related to chronic helminth infections may additionally decrease DM risk. On the other hand, a sedentary lifestyle and anemia have the potential to increase DM risk. Additionally, the mediating role of helminth-induced shifts in the gut microbiome composition remains to be determined [9].

A limited number of recently reviewed epidemiologic studies with inconsistent results investigated the cross-sectional association of different IDs, including lymphatic filariasis [10, 11], schistosomiasis [12], strongyloidiasis [13, 14], and soil-transmitted helminthiasis [15, 16] with DM or insulin sensitivity. In the present study, we followed up on these observations by studying the association of gastrointestinal tract infections due to helminths, intestinal

protozoa, and the bacterium *Helicobacter pylori* with glycated hemoglobin (HbA1c) concentration in school children in the frame of the "Disease, Activity and Schoolchildren's Health" (DASH) study in Port Elizabeth, South Africa [3, 17]. The study provided detailed information on physical activity, fitness, and socioeconomic status (SES) to consider as confounding factors on gastrointestinal tract infection status, and intensity of helminth infections to study a possible dose-response relationship; and on the longitudinal course of HbA1c upon selective anthelmintic treatment.

7.4 Methods

Ethics statement

Ethics approval was obtained from ethics committees in both Switzerland (EKNZ; reference no. 2014–179, approval date: 17 June 2014), and South Africa (study number H14-HEA-HMS002, approval date: 4 July 2014). Written informed consent from the parents/guardians of participating children as well as oral assent from the children were obtained prior to data collection.

Study setting and design

A total of 1,009 grade 4 children aged 9-14 years from eight non-fee paying primary schools were recruited in various parts of Port Elizabeth in the south-eastern part of South Africa, as described before [3, 17]. The study was part of the 2-year longitudinal DASH study that consisted of three cross-sectional surveys. In each of the cross-sectional surveys, children's gastrointestinal infections and other health parameters were assessed, including HbA1c, anthropometry, levels of physical fitness, cognitive performance, and psychosocial health. After each survey, helminth-infected children were treated with a single 400 mg oral dose of albendazole. In schools were the prevalence of helminth infection was 20% or above, all children were treated regardless of infection status according to guidelines put forth by the World Health Organization (WHO) [18]. Children with other infections (*Cryptosporidium* spp. and/or *Giardia intestinalis*) in combination with severe symptoms (e.g., bloody stool, diarrhea, abdominal pain, and any abnormal lung sounds) were referred to the nearest local health clinic for individual management.

The baseline cross-sectional survey took place in March 2015. The current study considers data from this baseline survey and the anthelmintic treatment follow-up examination in September/October 2015.

Inclusion and exclusion criteria

Grade-4 primary school children of the selected schools were included in the study. Children with severe clinical signs and symptoms (e.g., severe fever, severe headache, dizziness, nausea, skin rashes, seizures, and diarrhea) or reported serious health problems, such as Crohn's disease, liver or kidney diseases, or who participated in any other study were excluded.

Procedures

Questionnaires and interviews. Standardized questionnaires available in both English and local languages (Afrikaans and Xhosa) were used to determine the SES of the children and their families. Volunteers fluent in relevant languages were trained to conduct these in-person interviews.

Clinical and anthropometric assessment. Experienced nurses obtained a detailed medical history through physical examination of the whole body and evaluation of symptoms to assess current infections, anemia, jaundice, as well as signs and symptoms of protein energy malnutrition, general respiratory and gastrointestinal problems, allergies, and skin infections. Body temperature was measured using an infrared digital ear thermometer (TS7, Hi-Care International; Cape Town, South Africa). Blood pressure was measured once after the child had been seated for 5 min using validated oscillometric Omron® digital blood pressure monitor (Omron® M6 AC model; Hoofdoorp, Netherlands).

For the anthropometric measurements, shoes and sweaters were removed before standing on a digital weighting scale (Micro T7E electronic platform scale, Optima Electronics; George, South Africa). Body weight was measured once to the nearest 0.1 kg. Children's height was assessed with a Seca stadiometer (Surgical SA; Johannesburg, South Africa), whereby the child was standing with the back erect, heals touching the wall, and shoulders relaxed. Body height was taken to the nearest 0.1 cm.

Stool and urine sampling for assessment of gastrointestinal tract infection. A sample of at least 15 g of early morning stool from every participant was collected in a container and transferred to a laboratory of the Nelson Mandela University (NMU) in Port Elizabeth for diagnostic work-up. Stool samples were visually examined for *Taenia* spp. proglottids, signs of blood, mucus, and diarrhea. Duplicate 41.7 mg Kato-Katz thick smears were prepared from each stool sample [19] and examined under a microscope by two experienced laboratory technicians. The number of helminth eggs was counted and recorded for each species separately. Helminth egg counts were multiplied by a factor of 24 to obtain a proxy for

infection intensity, expressed as the number of eggs per gram of stool (EPG), which was then categorized into light, moderate, and heavy infections using readily available cut-offs offered by WHO [20]. For the detection of intestinal protozoa *C. parvum* and *G. intestinalis*, a Crypto-Giardia Duo-Strip® rapid diagnostic test (RDT) was performed on the stool sample [21]. For the discovery of the bacterium *H. pylori*, a Pylori-Strip® RDT was applied [22] (both tests are from CORIS, BioConcept; Gembloux, Belgium).

Children were also asked to provide a urine sample, which was transferred to the laboratory and analyzed on the same day. Visual inspection for macrohematuria was followed by testing for blood in urine using Hemastix® strips (Siemens Healthcare Diagnostics GmbH; Eschborn, Germany), as a proxy for *Schistosoma haematobium* infection. A point-of-care circulating cathodic antigen (POC-CCA) urine cassette test (Rapid Medical Diagnostics; Cape Town, South Africa) was used for the diagnosis of *Schistosoma mansoni* infection [23].

The infectious agents under the same taxonomy were grouped as trematodes (*S. mansoni* and *S. haematobium*), nematodes (*Ascaris lumbricoides*, *Enterobius vermicularis*, and *Trichuris trichiura*), intestinal protozoa (*G. intestinalis* and *C. parvum*), and bacteria (*H. pylori*) in the statistical analyses.

HbA1c measurement. HbA1c reflects plasma glucose concentrations over an 8- to 12-week period. It is used as a convenient diagnostic indicator for DM, as no fasting is required to measure it. HbA1c concentrations were obtained by using the POC instrument Afinion (Alere Inc. Waltham; Waltham, MA, USA), which is based on boronate affinity separation and the use of fluorescence quenching, with results available after 3 min. This method meets the generally accepted performance criteria for HbA1c, as defined by the U.S. National Glycohemoglobin Standardization Program (NGSP), with no interference from HbC, HbS, HbE, and HbD traits results. All test cartridges for the Afinion test belonged to the specific lot number. Test cartridges were stored at 4°C during the study and were removed from the refrigerator a maximum of 120 min before the test. The tests were run when the temperature of the cartridges were in their optimal range (15-25°C). Ambient room temperature was measured on each test day to assure absence of temperature effects on HbA1c test results as a means of quality control. Patients with HbA1c ≥6.5%, the recommended cut-off for diagnosing DM [24], were referred to DM care centers for confirmation and specific management.

Hemoglobin (Hb) measurement. Hb concentration was measured with the HemoCue® Hb 301 system (HemoCue®AB; Ängelholm, Sweden) and the results were considered to the nearest 0.1 g/l.

Covariates information

The SES was derived from housing characteristics and household assets (S1 Table). The SES score of the households was categorized as poorest, poor, and least poor using the scale by Filmer and Pritchett to disaggregate the distribution of the scores [25].

The age of individuals was grouped into five categories (8-9, 10, 11, 12 and >12 years), according to the age distribution of the population in the study. The body mass index (BMI) was calculated as kg/m^2 based on the measured height and weight. For physical activity, we used questionnaires on the frequency and duration of certain activities (how many days in a week the children were physically active for a total of at least 60 min, the traveling time from home to school, and numbers of exercising days and intensity of exercise in children's free time). The scores were summed up and equally categorized into tertiles: active, fair, and poor physical activity level according to the distribution of scores.

Cardiorespiratory fitness (VO_2 max) is the maximum rate of oxygen consumption, as measured during incremental exercise. We estimated the individual VO_2 max from the 20 m shuttle run test, which is the most widely used field test for determining cardiorespiratory fitness in children [26].

Statistical analysis

A complete case analysis was applied. Forty out of 882 participants at baseline moved or changed schools within the 6-month anthelmintic treatment follow-up, and hence, did not participate in the latter cross-sectional survey. Statistical analyses were performed with STATA version 14.1 (StataCorp; College Station, TX, USA). Statistical significance was defined as a two-sided p-value<0.05.

Descriptive statistics include counts, percentages for categorical variables and, means, and standard deviations (SD) for continuous variables. The categorization of DM status by sex is described according to the American Diabetes Association cutoffs for HbA1c. The baseline prevalence of the different gastrointestinal tract infections is presented for the different schools separately. The characteristics of covariates at baseline are presented stratified for infected and non-infected children. To assess the independent association between gastrointestinal tract infections and HbA1c measurement (treated as continuous numerical data) at baseline, linear mixed regressions models with random intercepts for

schools were computed. Models were *a priori* adjusted for factors previously shown to be associated with infections and glycemia or diabetes, and therefore with a potential role as confounders: age, sex, SES, Hb, height, weight, BMI, systolic and diastolic blood pressure, physical activity, VO_2 max, and body temperature. As a sensitivity analysis, we also omitted weight, BMI, physical activity, VO_2 max, anemia and blood pressure from the models as they are potential mediators of infection effects on glycemia or correlates of glycemia. All models were run (i) by adding each infection separately without excluding children with other infections; (ii) by adding each infection separately and excluding children with other infections; (iii) by adding all infection variables simultaneously and; and (iv) by adding groups of infections. We also assessed dose-response effects on HbA1c for infectious agents, especially *A. lumbricoides* infection, where data on intensity of infection was available. To assess the independent effect of anthelmintic treatment on changes in HbA1c level between baseline and the 6-month anthelmintic treatment follow-up among children from schools without lifestyle intervention and who were infected at baseline, linear mixed regression models with random intercepts for schools were built. Models were *a priori* adjusted for age, sex, SES, Hb, height, weight, BMI, diastolic and systolic blood pressure, physical activity, VO_2 max, and body temperature, considering information from both time points, as appropriate. Longitudinal models were re-run among subjects infected at baseline but not at follow-up, to differentiate between the effect of the anthelmintic treatment itself and the effect of resolved infection on change in HbA1c. Models were also run for children infected with nematodes and for children with any gastrointestinal tract infection separately.

7.5 Results

Complete data records including the baseline and 6-month anthelmintic treatment follow-up surveys were available from 842 children (Fig 1). Fig 2 shows the distribution of HbA1c at baseline and at the 6-month follow-up for the total study sample of 842 children irrespective of the intervention that they obtained. There was a small shift towards lower HbA1c levels at follow-up ($p<0.001$), reflecting the lifestyle intervention in some schools. The results of quality control tests underline the validity of the HbA1c data. First, HbA1c results did not depend on the day of examination ($p=0.222$), body temperature ($p=0.327$), or ambient temperature ($p=0.217$) (Fig S1a-c). Second, results from the weekly calibration with identical control 1 and control 2 are presented in S2 Table. At baseline, the overall mean HbA1c level of participants was 5.79% with SD of 0.25. The prevalence of prediabetes and diabetes according to baseline is presented in S3 Table. A high prevalence of preDM was

observed with 605 (71%) of children having preDM HbA1c levels. Three children (0.4%) exhibited HbA1c results ≥6.5% at baseline and were offered diagnostic follow-up for DM. The characteristics of the study population and its univariate association with HbA1c are presented in S4 Table.

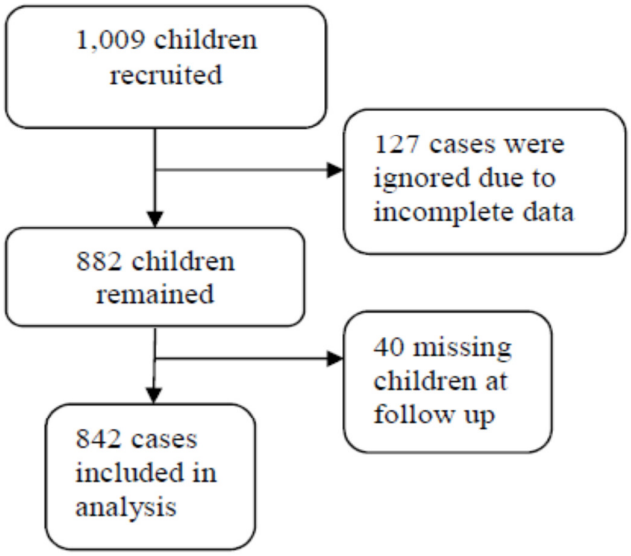

Fig 1. Children retained in the study sample for complete case analysis

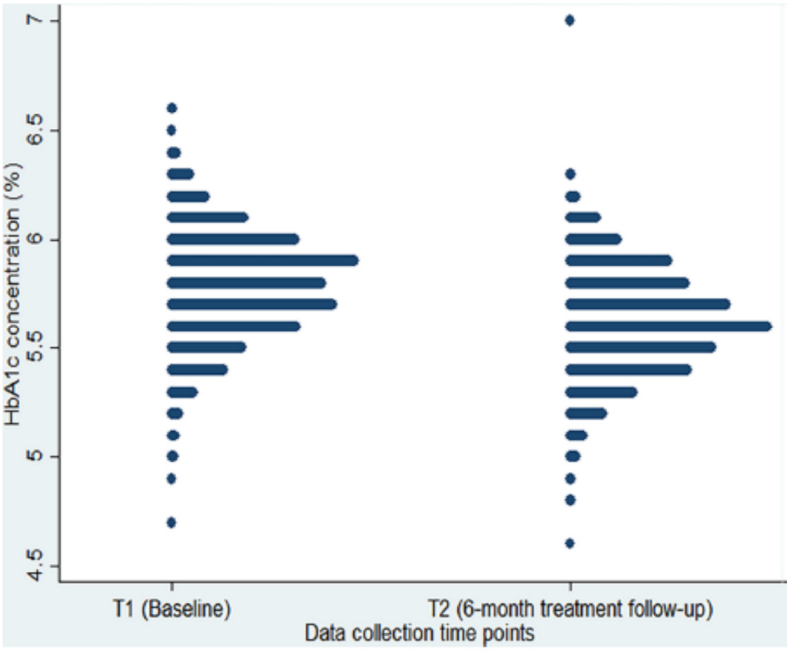

Fig 2. Distribution of HbA1c measured at baseline and 6 month follow-up in the total study sample, irrespective of the intervention obtained(N=842)

Table 1 shows the prevalence of gastrointestinal tract infections in the study schools at baseline. *H. pylori* was the predominant infection (416 children with a positive RDT result, 49.4%). At the unit of the school, the prevalence of *H. pylori* ranged from 27% to 62%.

The second most common infections were the soil-transmitted helminths *A. lumbricoides* and *T. trichiura.* Two out of eight schools showed very high prevalence of *A. lumbricoides* infection (62.5% and 74.1%), there was a moderate infection prevalence in a third school (25.9%), while the prevalence in the five remaining schools were below 5%. High prevalence of *T. trichiura* infection was observed in the same two schools where the prevalence of *A. lumbricoides* prevalence was high (66.7% and 67.9%, respectively), while the prevalence of *T. trichiura* was below 3% in the remaining six schools.

In all schools, infection rates were low to very low or even undetectable for intestinal protozoa (*Cryptosporidium* spp. 1-5%; *G. intestinalis* 6-17%), the nematode *E. vermicularis* (1-5%), and the trematodes *S. mansoni* (1-3%; detected by POC-CCA urine cassette test) and *S. haematobium* (0%).

Table 1. Baseline prevalence (%)[2] of gastrointestinal tract infections, stratified by schools

Schools and infection status	**School 1 N=89**	**School 2 N=168**	**School 3[1] N=81**	**School 4[1] N=102**	**School 5 N=82**	**School 6 N=84**	**School 7[1] N=143**	**School 8[1] N=93**	**Total N=842 100.0**
Nematodes	**n=1 1.1**	**n=133 79.2**	**n=67 82.7**	**n=4 3.9**	**n=1 1.2**	**n=4 4.8**	**n=42 29.4**	**n=9 9.7**	**n=261 31.0**
Ascaris lumbricoides	1.1	62.5	74.1	1.0	0	3.6	25.9	4.3	**n=211 25.1**
Trichuris trichiura	0	66.7	67.9	0	1.2	0	2.1	2.2	**n=137 16.3**
Enterobius vermicularis	0	1.2	3.7	2.0	1.2	2.4	2.8	4.3	**n=18 2.1**
Trematodes	**n=8 9.0**	**n=11 6.6**	**n=1 1.2**	**n=1 1.0**	**n=6 7.3**	**n=5 6.0**	**n=9 6.3**	**n=5 5.4**	**n=46 5.5**
Schistosoma mansoni	0	3.0	0	1.0	1.2	0	0.7	0	**n=8 1.0**
*Schistosoma haematobium**	9.0	3.6	1.2	0	6.1	6.0	5.6	5.4	**n=38 4.5**
Intestinal protozoa	**n=13 14.6**	**n=30 17.9**	**n=11 13.6**	**n=7 6.9**	**n=11 13.4**	**n=9 10.7**	**n=24 16.8**	**n=9 9.7**	**n=114 13.5**
Cryptosporidium	3.4	1.8	1.2	1.0	4.9	4.8	4.2	2.2	**n=21**

parvum									**2.4**
Giardia intestinalis	11.2	16.1	12.4	5.9	9.8	9.5	14.0	7.5	**n=96 11.4**
Helicobacter pylori	**n=62 69.7**	**n=111 66.1**	**n=50 61.7**	**n=53 52.0**	**n=35 42.7**	**n=23 27.4**	**n=42 29.4**	**n=40 43.0**	**n=416 49.4**

[1] Schools without intervention related to health education, nutrition, and physical activity

[2] A detailed description of infections in the 8 schools is presented in our previous paper [27]

N Number of children in each school

n Number of infected children in each school and parasite group

**S. haematobium* infections only detected with Hemastix® strips

Table 2 simply compares the characteristics of participants with and without a specific gastrointestinal tract infection. Except for *H. pylori*, the proportion of children with low SES was higher among infected children compared to their non-infected counterparts. Infections with nematodes and *G. intestinalis* were more common in males, whereas *C. parvum* infection was more common in females. Infected children were, on average, older than their non-infected peers. Nevertheless, children with an *A. lumbricoides*, *T. trichiura*, and *E. vermicularis* infection had lower height, weight, and BMI compared to non-infected children. However, children infected with *A. lumbricoides*, *T. trichiura*, and *H. pylori* reported higher physical activity, but did not differ with regard to cardiorespiratory fitness. Concerning anemia and HbA1c, no clear pattern of association was evident from the univariate analysis.

Table 2. Distribution of characteristics of participants at baseline, by presence or absence of specific gastrointestinal tract infections

Covariates	*A. lumbricoides*		*T. trichiura*		*E. vermicularis*		*S. mansoni*		*S. haematobium*		*C. parvum*		*G. intestinalis*		*H. pylori*	
	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
Low SES, N, %	189, 30.0	147, 70.0	218, 32.6	118, 68.2	326, 39.6	10, 55.6	332, 39.8	4, 50.0	316, 39.3	20, 52.6	329, 40.0	7, 33.3	291, 39.0	45, 46.9	172, 40.4	164, 39.4
Female, N, %	322, 51.4	94, 45.5	342, 51.4	74, 42.7	409, 50.1	7, 38.9	412, 49.9	4, 50.0	392, 48.8	24, 63.1	401, 48.8	15, 71.4	374, 50.1	42, 43.8	205, 48.4	211, 51.4
Age, years N, mean,SD	631, 10.7, 0.972	211, 11.2, 0.865	669, 10.7, 0.987	173, 11.2, 0.789	824, 10.8 0.969	18, 10.9, 1.017	834, 10.8, 0.970	8, 11.6, 0.721	804, 10.8, 0.953	38, 11.2, 1.239	821, 10.8, 0.972	21, 10.8, 0.905	746, 10.8, 0.964	96, 11.1, 0.994	426, 10.8, 1.012	416, 10.9, 0.926
Height, cm, N, mean, SD	631, 133.8 7.083	211 131.5, 6.830	669, 133.8, 6.933	173, 130.8, 7.164	824, 133.3, 7.063	18, 130.0 7.547	834, 133.2, 7.064	8, 139.4, 6.931	804, 133.1, 7.034	38, 134.7, 8.045	821, 133.1, 7.039	21, 134.8, 8.756	746, 133.2, 7.067	96, 133.3, 7.257	426, 133.7, 7.214	416, 132.8, 6.929
Weight, kg, N, mean, SD	631, 31.3, 8.048	211 28.1, 5.580	669, 31.3, 7.901	173, 27.4, 5.463	824, 30.6, 7.660	18, 26.9, 4.662	834, 30.4, 7.634	8, 34.1, 5.944	804, 30.5, 7.668	38, 30.9, 6.747	821, 30.4, 7.596	21, 32.7, 8.615	746, 30.5, 7.628	96, 30.5, 7.650	426, 30.0, 7.938	416, 30.9, 7.276
BMI, kg/m^2, N, mean, SD	631, 17.3, 3.254	211 16.1, 2.102	669, 17.3, 3.213	173, 15.9, 1.930	824, 17.0, 3.066	18, 15.8, 1.626	834, 17.0, 3.048	8, 17.6, 3.108	804, 17.0, 3.084	38, 16.9, 2.168	821, 17.0, 3.057	21, 17.7, 2.602	746, 17.0, 3.054	96, 17.0, 3.010	426, 3.2, 3.186	416, 2.9, 2.898
VO_2 max, ml/kg/min, N,mean, SD	631, 45.9, 5.080	211 45.5, 5.098	669, 45.8, 5.099	173, 45.8, 5.055	824, 45.8, 5.048	18, 46.6, 6.729	834, 45.8, 5.073	8, 46.4, 6.805	804, 45.9, 5.081	38, 44.3, 5.210	821, 45.9, 5.081	21, 43.8, 5.050	746, 46.1 5.027	96, 45.5, 5.562	426, 46.0, 5.375	416, 45.9, 4.781
Low physical activity, N,%	250, 39.6	56, 26.5	271, 40.5	35, 20.2	297, 36.0	9, 50.0	302, 36.2	4, 50.0	292, 36.3	14, 36.8	298,3 6.3	8, 38.1	274, 36.7	32, 33.3	176, 41.3	130, 31.3

Hb, g/l N, mean, SD	631, 12.3, 9.482	211 11.9, 9.158	669, 12.3, 9.629	173, 11.9, 8.367	824, 12.2, 9.475	18, 12.1, 10.820	834, 12.2, 9.491	8, 12.3, 11.179	804, 12.2, 9.474	38, 12.5, 9.887	821, 12.2, 9.460	21, 12.3, 11.240	746, 12.2, 9.635	96, 12.3, 8.417	426, 12.3, 9.594	416, 12.2, 9.389
HbA1c %, N, mean, SD	631, 5.8, 0.245	211 5.7, 0.255	669, 5.8, 0.243	173, 5.7, 0.270	824, 5.8, 0.249	18, 5.7, 0.266	834, 5.8, 0.250	8, 5.9, 0.225	804, 5.8, 0.250	38, 5.8, 0.229	821, 5.8, 0.249	21, 5.8, 0.264	746, 5.8, 0.245	96, 5.8, 0.284	426, 5.8, 0.246	416, 5.8, 0.253

N Number of children

The results from the multivariable linear regression models of the cross-sectional association of single or grouped infections with HbA1c are presented in Table 3. We observed a positive association between *H. pylori* infection and HbA1c, irrespective of adjustments for other infections (β=0.040; 95% confidence interval (CI) 0.006 - 0.074). No significant association of HbA1c with any other infectious agent or infection group was observed. Omitting covariates from the multivariable regression models that are potential mediators of infection effects on glycemia (physical activity; physical fitness; weight, BMI, anemia) or correlated outcomes (blood pressure) did not materially alter the results presented for the fully adjusted models (S5 Table). Excluding children with diabetes at baseline or at the 6-month anthelmintic treatment follow-up did not materially alter the findings (S6 Table). In addition, we were not able to show a statistically significant dose-response relationship between intensity of *A. lumbricoides* and *T. trichiura* infection and HbA1c levels, albeit adjusted HbA1c levels were highest in children with most intense infections (S7 Table).

Table 3. Adjusted associations of infection with HbA1c at baseline

Table 3a: Single Infections and infection Groups	**All with respective infection[1]**			**Only respective infection[2]**			**Mutually adjusted for other infections or groups[3]**		
	N	**β***	**95% CI**	**N**	**β ***	**95% CI**	**N**	**β***	**95% CI**
Nematodes	842	-0.018	-0.069 – 0.032	343	-0.039	-0.115 – 0.037	842	-0.027	-0.079 – 0.024
A. lumbricoides	842	-0.021	-0.070 – 0.029	307	-0.039	-0.133 – 0.055	842	-0.029	-0.080 – 0.023
T. trichiura	842	0.000	-0.060 – 0.060	280	0.061	-0.208 – 0.330	842	0.002	-0.061 – 0.066
E. vermicularis	842	-0.053	-0.164 – 0.058	284	-0.079	-0.251 – 0.093	842	-0.057	-0.168 – 0.054
Trematodes	842	0.013	-0.058 – 0.084	296	0.012	-0.097 – 0.120	842	0.012	-0.059 – 0.083
S. mansoni	842	0.033	-0.132 – 0.199	278	0.069	-0.387 – 0.525	842	0.029	-0.137 – 0.195
S. haematobium	842	0.008	-0.070 – 0.086	295	0.011	-0.102 – 0.123	842	0.006	-0.072 – 0.084
Protozoa	842	-0.006	-0.052 – 0.041	283	-0.126	-0.314 – 0.062	842	-0.005	-0.052 – 0.042
C. parvum	842	-0.020	-0.122 – 0.083	283	-0.121	-0.310 – 0.067	842	-0.030	-0.133 – 0.074
G. intestinalis	842	0.005	-0.045 – 0.055	305	0.001	-0.093 – 0.095	842	0.006	-0.045 – 0.057
H. pylori	**842**	**0.040**	**0.006 – 0.074**	488	0.041	-0.003 – 0.085	**842**	**0.041**	**0.007 – 0.075**

Table 3b: Nematode infections	**All with respective infection[1]**		
	N	**β**	**95% CI**
Only nematodes	842	-0.057	-0.124 – 0.011
Nematodes and other infections	842	0.013	-0.046 – 0.070
Only other infections	842	0.016	-0.024 – 0.055

* Beta coefficients reflect the adjusted mean difference HbA1c (%) between children with and without the respective infection. Differences that are statistically significantly different ($p<0.05$) are marked in bold.

[1]Single and group infection models as well as nematode infection models are adjusted schools, for age, sex, socioeconomic status (SES), hemoglobin (Hb) level, height, weight, BMI, systolic and diastolic blood pressure, physical activity, physical fitness, body temperature on the day of the HbA1c test

[2]children with other infections are excluded from this analysis

[3]Mutually adjusted models include either all single infections or all infection groups; *H. pylori* is included in single infection and infection group models

Results pertaining to the association between albendazole treatment and change in HbA1c level at the 6-month treatment follow-up are presented in Table 4. The analysis is restricted to children from schools not subjected to lifestyle interventions given the observed slight decrease in HbA1c in the total study sample. Furthermore, only children with any infection or with nematode infection at baseline, respectively, were included. The regression analyses point to statistically non-significant increases in HbA1c concentrations at the 6-month treatment follow-up. The findings from multivariable regression model excluding covariates that could be potential mediators of infection effects on glycemia or correlated outcomes (weight, BMI, anemia, physical activity, physical fitness, blood pressure) point to generally weaker and still statistically non-significant results but in the subjects with a nematode infection at baseline, but without any infection at follow-up showing decreased estimate average change in HbA1c (S8 Table).

Table 4. Adjusted[1] estimate of average change in HbA1c (follow-up minus baseline) among children infected at baseline and visiting schools without lifestyle intervention

Infections exposures	N	Estimate average change in HbA1c (%)	95% CI
Nematode infections			
All subjects with a nematode infection at baseline, adjusted for the presence of infection of any type at baseline and follow-up	414	0.049	-0.018 – 0.117
Subjects with a nematode infection at baseline, but without any infection at follow-up	217	0.025	-0.008 – 0.108
Any infection			
All subjects with any infection at baseline, adjusted for the presence of infection at follow-up	260	0.070	-0.008 – 0.148
Subjects with any infection at baseline, but without any infection at follow-up	103	0.054	-0.055 – 0.164

[1]All models were adjusted for schools, age, sex, socioeconomic status (SES), hemoglobin (Hb) level, weight, height BMI, physical activity, VO_2 max, and body temperature, systolic and diastolic blood pressure at baseline and follow-up

7.6 Discussion

To our knowledge this is the first investigation examining the cross-sectional association of a broad spectrum of gastrointestinal tract infections with glycemia in school-aged children and assessing the impact of anthelmintic treatment on the change in HbA1c values. We observed a positive association between *H. pylori* infection and HbA1c, while no statistically significant relationship was observed with any other type of infection.

Some animal experiments [28] and human epidemiologic studies [10, 11, 13, 15] have shown helminth infections to lower the blood sugar level and inhibit the development of type 1 DM as well as type 2 DM. An inverse relationship between lymphatic filariasis and both type 1 and type 2 DM was reported from India [10, 11]. Having a previous schistosome infection exhibited a strong protective effect against DM in the People's Republic of China [12]. *Strongyloides stercoralis* infection seemed to be associated with a reduced risk of type 2 DM in adult Australians [13]. Soil-transmitted helminth infections were linked with an improvement of insulin sensitivity in Indonesia [15]. Diabetic patients in Turkey were found to have a lower prevalence of parasitic disease than their healthy counterparts [16]. In contrast, a positive association was found between *S. stercoralis* infection and DM in Brazil, where it was also found that such infections were associated with a high mortality risk among poorly controlled DM patients [14]. A study conducted by Hakim and colleagues reported a high rate of *G. intestinalis* infection among DM patients [29]. For trematode infections, positive association with HbA1c concentrations were reported from several studies [12, 30, 31]. The cross-sectional nature of these studies precludes casual inference.

H. pylori is one of the most common human pathogens causing gastrointestinal inflammation. Potential underlying mechanisms linking *H. pylori* infection and HbA1c levels and DM may include a disturbance of glucose and lipid absorption by the inflamed gastrointestinal tissue. *H. pylori* infections may also alter host metabolic homeostasis by affecting appetite regulation and energy expenditure through altered balance of ghrelin and leptin secretion, leading to over-eating and metabolic syndrome pathogenesis. The mediating role of gut microbiota alterations remains unknown [32]. The reported associations between *H. pylori* infection and DM remain inconsistent. The positive association reported among school children in the present study corroborates findings from two large cross-sectional national surveys conducted by Chen and Blaser in American population samples (one aged ≥18 years and one aged ≥3 years) and a Taiwanese study in adults, which all found that *H. pylori* infections were associated with higher mean HbA1c levels [33, 34]. Several smaller

outpatient clinic or hospital based studies in Turkey, Pakistan, and Qatar among adults aged 18 years and above showed a higher prevalence of *H. pylori* infection in diabetic patients than non-DM control groups [35-37]. Other studies failed to find a positive association between *H. pylori* and HbA1c or DM [38-40].

In fact, DM patients were found to have higher rates of *H. pylori* eradication therapy according to national health insurance data from Taiwan. *H. pylori* eradication treatment success was found to be lower in DM compared to non-DM patients [41, 42]. Future intervention studies for the treatment of *H. pylori* should systematically consider changes in glycemia to shed light on the potential etiologic role of *H. pylori* in DM development. Some studies indicated an improvement of mean HbA1c and insulin resistance in patients with type 2 DM after *H. pylori* treatment [43, 44].

We did not observe a statistically significant increase in HbA1c after anthelmintic treatment with albendazole in children harboring nematode infections at baseline, possibly as a result of sample size limitations. The observed direction of the effect is in line with the reported shift towards a Th2 response in helminth-infected individuals. A number of clinical trials with helminth or helminth antigen therapy have reported promising results in inflammatory bowel diseases [45, 46], multiple sclerosis [47], rheumatoid arthritis [48]. After deworming, which triggers several hyper-inflammatory processes and shifts immune responses from Th2 to Th1, groups of children treated with either albendazole or mebendazole (against soil-transmitted helminthiasis) or praziquantel (against schistosomiasis) had a higher positive response to the skin-prick test and allergy related symptoms [49, 50]. Nevertheless, other studies emphasized that anthelmintic treatment did not have an effect on clinical eczema and asthmatic severity scores [51, 52].

Given that in our study the highest increase in HbA1c after albendazole treatment was observed in children with non-nematode parasite infection, additional research is needed to understand the effect of the anthelmintic drugs on human glucose metabolism. Yet, our results are aligned with the first publication from a randomized placebo-controlled trial in Indonesia, which showed no effect of albendazole treatment on insulin resistance [53].

Our study has several strengths. First, the study population exhibited sufficient prevalence range for at least some of the infectious agents investigated to allow for efficient interrogation of the study objective. Second, the detailed characterization of children allowed us to assess independent associations of parasite infections with glycemia and limiting residual confounding. To analyze the SES of study participants, we chose multiple

correspondence analysis (MCA) based on household characteristics and assets ownership over more traditional methods thereby minimized measurement error related to the different calculation methods of income and consumption, recall bias, and seasonal variation of income and expenditure. Third, we used internationally certified HbA1c testing (Alere Technologies), regularly calibrated with standard control procedure. Ehehalt et al. showed that the measurement of HbA1c was a reliable criterion for children and adolescents to diagnose the onset of childhood type 1 DM [54]. In addition, the POC HbA1c test is an accepted screening instrument for pre-DM and type 2 DM [55, 56]. We carefully evaluated potential measurement error in HbA1c in the light of the observed high prevalence of pre-DM. We demonstrated the absence of correlations with external temperature, body temperature, and examination date. In addition, all models were adjusted for the concentration of Hb, a potentially important confounder, which was assessed with the widely used HemoCue Hb 301 system.

We also acknowledge some limitations of our study. Reverse causation remains a problem related to the cross-sectional nature of our main analysis. The low prevalence for some infections limited statistical power for the analyses. The association between *H. pylori* infection and HbA1c is no longer statistically significant if the p-values are adjusted for the number of infections investigated (n=8). Additionally, the co-infections [27] may in part mask opposite effects of different parasites on HbA1c. Examining only one stool sample has a low diagnostic accuracy due to the day-to-day and intra-specimen variation in helminth egg output. To partially remedy this shortcoming, test specificity was increased by preparing duplicate Kato-Katz thick smears from each stool sample. We observed a very high rate of prediabetes in the children studied, which may limit the generalizability of the observed associations. Despite the fact that the Alere HbA1c testing is minimally affected by hemoglobinopathies, we cannot assess any influence in the absence of genotyping results. Selection bias related to the complete case analysis approach cannot be excluded but the very high participation rate at baseline and the 6-month anthelmintic treatment follow-up (5% drop-out rate), and the relatively low rate of children not providing stools (15%) are unlikely to have substantially altered the results.

In conclusion, the positive cross-sectional association of *H. pylori* infections with glycemia is consistent with a potential role of this highly prevalent bacterium in DM in LMICs. The direction and causality of the association warrants further scientific inquiry in the context of longitudinal studies and biobanks that focus on specific parasites and integrate

immunity as well as other biomarkers to improve mechanistic understanding of parasite-glycemia associations and the potential impact of deworming programs on DM prevalence.

7.7 References

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Supporting information

S1a Fig. Plot of individual baseline HbA1c results versus date of examination in all schools

S1b Fig. Plot of individual baseline HbA1c results versus body temperature in all schools

S1c Fig. Plot of individual baseline HbA1c results versus ambient temperature in all schools

S1 Table. Multiple Component Analysis (MCA) of SES of the participants

S2 Table: HbA1c measurements in control probes integrated into baseline and follow up assessment

S3 Table. Distribution of prediabetes and diabetes based on HbA1c cutoff[1] at baseline, by gender

S4 Table. Demographic profile of participants and HbA1c assessment

S5 Table. Adjusted association of helminth infections and HbA1c measurement at baseline, omitting adjustment for potential mediators and correlated outcomes

S6 Table. Adjusted association of helminth infections and HbA1c measurement at baseline - DM cases excluded

S7 Table. Adjusted association of *A. lumbricoides* and *T. trichiura* infection intensities and HbA1c measurement at baseline

S8 Table. Adjusted estimate of average change in HbA1c (follow-up minus baseline) among children infected at baseline and visiting schools without lifestyle intervention, omitting adjustment for potential mediators and correlated outcomes

Chapter 8

Discussion

8.1 Overview of discussion

This PhD thesis pertains to dual burden of diseases, i.e. chronic diseases with communicable diseases between DM and NTDs, particularly on dengue viral infections and helminth infections. To have a true understanding of epidemiological association and associated risk factors between these particular diseases, we performed a systematic literature review (and meta-analyses) with the data from published articles. The findings from the review have been verified by epidemiological field studies.

The justifications of this thesis are as follows: DM prevalence is a growing epidemic in both developed and developing world. Dengue is currently one of the most re-emerging tropical diseases and dengue outbreak increased in recent years. Dengue, nowadays not only affects to children but also adults. However, there is very little knowledge on the association between DM and dengue viral infections. Our systematic literature highlighted the importance of comorbidities of DM in the development of severe dengue. The current information of relevance on these diseases associations was also updated and we identified the needs for the further research and comorbid patient care.

A number of epidemiological studies and clinical trials reported that helminthes were protective against autoimmune diseases. Animal studies also confirmed that experiment with helminth eggs in mice slowed down the onset of developing type 1 DM, but the studies in human setting and type 2 DM is still lacking and the methodology of previously conducted studies did not adequately explain the underlying mechanism. The helminth-DM literature review summarized the current evidence of the effects of each helminth on different types of DM with their comorbid associations and set the stage for additional studies. This understanding was applied in our field studies in Laos PDR and South Africa. Studying comorbidity of NCD and NTDs is novel in both children and adults population. Our studies also gave novel results on comorbid relationships and gave a new insight into the single and dual disease burden in both countries from DM perspective. Moreover, we also assessed the regional differences in

prevalence of different helminth infections (and parasite infections in the South African study) as well as the DM status and DM risks of population. We also observed in more of a pilot setting the possible linkage between helminth infections and the blood sugar status after anthelmintic treatment. This will also provide significant impact in terms of developing potential novel therapeutic strategies (either a vaccine or a drug using helminth related compound or a substitute) aimed at preventing and/or delaying the disease onset and/or decreasing the severity of DM. In addition, the studies conducted in Lao PDR and South Africa created a reasonable awareness related to dual disease burden to health policy makers in helminth endemic countries, which are currently implementing the nationwide mass deworming program for continuation or discontinuation of deworming program.

8.2 Discussion of the main findings

8.2.1 Association between diabetes mellitus and dengue viral infections

Dengue is currently concerned as the most emerging mosquito-transmitted tropical disease in every geographical region of the world, particularly in South Asia, South East Asia and Latin America (Brazil and Mexico) (Bhatt et al., 2013, Murray et al., 2013). It is linked with the dynamics of globalization (Gubler, 2011), international traveling and trading (Russell et al., 2009), global population growth and rapid urbanization, socio-economic constraints on control measures (Gubler et al., 2001, Astrom et al., 2012, Brunkard et al., 2008), climate change (Astrom et al., 2012), and the evolution of viral virulence (Jarman et al., 2008, Rico-Hesse, 2003). At the same time, many of the tropical countries begin to encounter the epidemiological transition, adding burden of NCDs such as DM, cardiovascular diseases, cancer, chronic obstructive pulmonary disease to the overall mortality rate along with ageing of the population (Porta MS., 2008). The co-morbidities of dengue with NCDs in these regions are expected to be increasing and the understanding of the relevance of DM in the development of severe dengue is fundamental in order to improve the clinical monitoring and interventions.

Associated risk factors

The findings of our DM-dengue study showed that patients with dengue fever and DM comorbidities seem to be at higher risk of developing complications and/or severe dengue compared to those without comorbid conditions although this result was based on very limited data. Some studies have postulated that in adults, non-communicable comorbidities and other

underlying medical conditions such as cardiovascular diseases, endocrine diseases, allergies, hematological diseases, chronic hepatopathy, recipients of solid organ transplant, chronic renal insufficiency, autoimmune disorders, respiratory diseases, stroke and also the condition of being old age may have a role in predisposing individuals to the severe forms of dengue (Lye et al., 2010, Toledo et al., 2016). The data of Cuban epidemic dengue outbreaks also expressed that co-presentation between dengue and DM and having diabetes mellitus was a risk factor contributing to the development of dengue haemorrhagic fever (Guzman, 2012). A number of studies revealed that the effects of nutritional status on dengue disease outcome have been controversial. Some studies found that patients with excessive body weight or obesity have increased risk for severe dengue fever (Pichainarong et al., 2006, Junia et al., 2007) as the physiological reaction of a person with a high body mass are intrinsically more likely to leak the fluid from blood vessels to surrounding tissues and organs. Other studies mentioned that malnutrition is a protective factor due to suppressed immune activation in malnourished children (Nguyen et al., 2005, Thisyakorn and Nimmannitya, 1993). Among different serotypes of dengue viral infection, infection with one serotype gives lifelong immunity to that type, but only short term immunity to the other serotypes. Due to seasonal variation, therefore, subsequent infection with a different type increases the risk for severe disease and complications (Rodenhuis-Zybert et al., 2010). A Pakistani study conducted in the Lahore also showed a significant association between dengue and diabetes particularly among middle to elderly participants (Aamir et al., 2015).

Clinical management of dengue in DM patient

Due to no specific treatment for dengue viral infection (Simmons et al., 2012, Halstead, 2007, WHO, 2009) and the pathophysiological mechanism of increasing vascular permeability and severe bleeding, the standard therapy of clinical management of severe cases of dengue is fluid replacement, both orally and intravenously (Rosenberger et al., 2016) with intensive monitoring of plasma leakage and supportive care. However, excessive fluid replacement might lead to hypervolemia, pulmonary edema and respiratory distress. This clinical aspect is very important in elderly patients, due to their respiratory-cardiovascular and renal compliance (WHO, 2009, Mandell GL, 2005). In addition, according to Hasanat and colleagues study, glucose intolerance is frequently associated with dengue fever in its early course of dengue infection (Hasanat et al., 2010) and therefore, clinicians should be aware using of dextrose contained infusions as fluid replacement in dengue fever.

Dengue vaccine

In late 2015, the first dengue vaccine, Dengvaxia (CYD-TDV), a live recombinant tetravalent vaccine with a 3 series of doses scheduled on a 0,6,12 month aiming to provide the long term immunity to all serotypes, was introduced by Sanofi Pasteur pharmaceutical company in five Latin American countries (Villar et al., 2015). With scientifically evidenced efficacy of the vaccine with fewer hospitalization among virologically confirmed dengue cases, WHO considers dengue vaccines to be an integrated part of the Global dengue prevention and control strategy (2012-2020) (WHO, 2012) and recommends the dengue endemic countries to consider introducing the dengue vaccine for individuals aged between 9 and 45 years in order to reduce the burden of dengue. Other additional potential vaccines are now under evaluation in clinical trials (Thisyakorn and Thisyakorn, 2014). The WHO Initiative for Vaccine Research (IVR), in collaboration with a wide range of partners, aims to facilitate the development and future introduction of safe, effective and affordable dengue vaccines in epidemiologically burden dengue area.

Limitations of our study

Our findings from the systematic literature review and meta-analyses have limitations. Some retrieved studies did not mainly focus on DM and dengue as the exposures and outcomes and most of the clinical data information relevant comorbidities were usually underreported in the articles which made it difficult to retrieve these information from the data provided from the studies and to summarize and conclude the results. Additionally, the studies in our review were mostly case-control and retrospective in nature and thus, there is likely to be a selection bias of research participants either in-patients or out-patients, who lived nearby the health facilities or hospitals. The retrieved studies did not also represent or recruit non-severe dengue cases that were treated at the general practitioners. This also raised the variability of studies and generalizability of study population.

8.2.2 Association between DM and helminth infections

In the previous sections (chapter 5, chapter 6 and chapter 7), the discussion of each research study findings and outcome has been provided. In this chapter, a global relevant perspective on results of the association between DM and heminth or parasite infections is given

Effects of helminthes, parasites, bacterial and viral infections on DM status

In our studies, we found the different prevalence of helminth infections in the study area (different provinces in LADUBU study and different schools in South African study). In LADUBU study, the food-borne trematodes were prevalent across all provinces. In South Africa, STHs were more common and the rate of infections varies from schools to schools. We found a significant positive association between *Taenia* infection and DM status measured by HbA1c blood concentration in Lao PDR study. *T. solium* infection has been widely found in China, Nepal, India, Philippines, Indonesia, Thailand, Cambodia, Vietnam and Lao PDR (Conlan et al., 2008, Willingham Iii et al., 2010). *T. saginata* has only been reported in southern Laos (Sayasone et al., 2009) and *T. solium* and *T. saginata* have been common in northern Laos (Eom et al., 2009). The prevalence of *Taenia* was more frequent in pigs than human. Even taeniasis itself or cysticercosis caused by *T. solium* is a major NTD and substantial work for elimination programs in various geographical regions around the world exists, the parasites still remains. In taeniasis, the main risk factor of infection is insufficiently cooked meat consumption (either pork or beef). Among *Taenia* spp, *T. solium* infection can mainly cause major health problems and elimination programs of *T. solium* have been ineffective in endemic countries, due to insufficient integrated surveillance in both human and animals (Willingham Iii et al., 2010). Our findings of *Taenia* infection being positively associated with the blood sugar levels is confirmed in additional studies that integrate biomarkers to improve mechanistic understanding, screening and elimination of taenia infection in DM patients and vice versa. DM control in persons with taenia infection becomes clinically relevant.

Our South African study revealed a positive association between *Helicobactor pylori* infection and HbA1c measurements. *H. pylori* infection is widespread in South Africa and is present in 70% of gastric ulcer and 90% of duodenal ulcer and carcinoma of stomach in advanced stage (Figueiredo et al., 2005). Multidrug resistance is also identified as a major cause of treatment failure in South Africa (Tanih et al., 2010). Growing research evidence describes that *H. pylori* infection is associated with DM via *H. pylori* induced gastritis that affects gastric related hormones and inflammatory cytokines, leading to insulin resistance; however, the relationship remains debatable due to complicated autoimmune inflammatory mechanism behind. It was shown that *H. pylori* treatment or eradication in DM patients resulted in decreased mean HbA1c (Zojaji et al., 2013) or fasting insulin HOMA-IR levels (Gen et al., 2010) , while other studies showed no effect (Wada et al., 2013, Vafaeimanesh et al., 2013). However, Zojaji

and colleagues suggested that it could be beneficial to inspect the *H. pylori* infection among the individuals at high risk of DM (Zojaji et al., 2013).

The factors that influence the dual disease relationship include helminth or parasite species, host genetics, timing, burden and chronicity of helminth infections (Cooper, 2009). Some studies suggested that the parasite related protective immune effects could be the most effective in early life to combat the autoimmune diseases (Ndibazza et al., 2012, Pelosi et al., 2005).

Other infectious agents such as bacteria or viruses are also associated with both type 1 or type 2 DM as either an increased or a decreased risk. Studies found a significant association between enterovirus infection (Yeung et al., 2011), Cytomegalovirus (Yoneda et al., 2017) and both type 1 and related autoantibodies. The German population study also reported that recurrent viral respiratory tract infections in the first 6 months of life were associated with an increased chances of having type 1 DM by their age of 8 (Beyerlein et al., 2016). Other reports presented a weak association between mumps and type 1 DM (Saad et al., 2016) but other studies outlined no evidence of viral infections with type 1 DM development (Cinek et al., 2014, Lee et al., 2013). In animal studies, a link has been found between H1N1 as well as Coxsackie B virus and type 1 DM (Qi et al., 2017). Moreover, it is known that hepatitis C (Antonelli et al., 2014, Desbois and Cacoub, 2017), Herpes virus (Pompei, 2016), endotoxins (Min and Min, 2015) could be associated with type 2 DM. Again, some bacterial infections also have a relationship with DM. Staphylococcal infections, Streptococcal infections, and *Klebsiella pneumoniae* are common infections among DM patients. Uncontrolled DM is also associated with increased risk of TB (Leung et al., 2008) but not in other study (Leegaard et al., 2011). A study in Tanzania remarked that transient hyperglycemia is frequent during clinical course of tuberculosis, and further DM confirmation and diagnosis is essential after tuberculosis treatment (Boillat-Blanco et al., 2016). Finally, a bacterial infection called melioidosis has been strongly associated with DM. Therefore, knowledge of melioidosis and DM association is quite important for endemic countries such as Southeast Asia and Northern Australia (Cheng and Currie, 2005, Currie, 2015) .

Co-infections and multiparasitism

Multiparasitism or co-infections were also very common in both of our studies. Multiparasitism, also known as polyparasitism, can be defined as the concurrent infestation with

two or more parasite species in high frequencies and mostly intestinal forms (Steinmann et al., 2010). Though it is an important public health concern in tropical and subtropical countries, it is often being neglected. The assessment of multiparasitism is always a challenging issue due to the necessity of sensitive broad-spectrum diagnostic approaches and collecting multiple biological samples for each and specific helminth. Besides, the effects of multiple species infections on disease outcome in hosts are usually multiplex due to a potential synergistic or antagonistic effect between parasites. The co-infections can also alter the host susceptibility, transmission risks, and clinical symptoms. Additionally, the effects of associations are further affected by a diversity of potential risk factors such as the status of socioeconomic demography, environmental exposure and immunological reactions.

Typically after treatment, reinfection rate especially STH is high and occur rapidly if the exposure is not changed. E.g. the reinfection rate 3 months after the anthelminthic treatment was 26% for *A. lumbricoides*, 36% for *T. trichiura* and 30% for hookworm (Jia et al., 2012). The reinfection rates were *T. trichiura* (37.2%), for *A. lumbricoides* (34.6 %) and for hookworms (25.0%) after 18 weeks post-treatment in Tanzania (Speich et al., 2016). Re-infection rate of *S. stercoralis* was found among one third of ivermectin treated schoolchildren (Khieu et al., 2014). In Thailand, re-infection rate of *O. viverrini* after one year with Praziquental treatment was between 54.8% and 94.0% (Sithithaworn and Haswell-Elkins, 2003). The main reasons were due to the high frequency of raw and insufficiently cooked fish consumption and inadequate sanitation facilities the transmission. In our longitudinal South African study, we also found that the 2 schools which had a higher infection rate were still infected and the re-infection rate at follow up was almost as similar as baseline, 6 months after deworming with Albendazole.

Gut microbiome, obesity and metabolic dysfunction

The role of gut microbiome is crucial in controlling human metabolic metabolism by regulating the host genes (Tilg and Kaser, 2011). In murine studies, the microbiome affect the host energy expenditure, immune and inflammatory mechanisms via several pathways such as fasting induced adipose factor in central regulation of energy metabolism (Kim et al., 2010), affecting skeletal muscle fatty acid oxidation that control cellular energy status (Backhed et al., 2007), synthesizing hydrolases which influences host energy storage (Backhed et al., 2005). However, other experiments report that obesity can influence the composition of gut microbiota (Ridaura et al., 2013). Since there is an increased risk of developing type 2 DM in obesity, the

microbiome might also influence type 2 DM through increased acetate and decreased butyrate production which cause insulin resistance leading to low grade inflammation in the gut, as shown in human studies (Forslund et al., 2015). A study with more than 7,000 children also showed the link of probiotics usage during the first month of life to a lower risk of islet autoantibodies, suggesting that the gut microbiome may also play a role in type 1 diabetes mellitus (Uusitalo et al., 2016). The human microbiomes contain exponentially more genes than human genes, and those microbial genes produce molecules that affect human physiology when they are disordered. Modulation of microbiota through diet, pre- and probiotics, antibiotics, surgery, and fecal transplantation has a major impact the obesity epidemic (John and Mullin, 2016). A proper understanding of environmental influences on the microbiota and the consequences on metabolic-inflammatory diseases become a key aspect.

Helminthes and metabolic syndrome/parameters

Metabolic syndrome (MetS) has reached epidemic proportions across the globe and place huge burdens on the health systems of both economically developed and developing societies (Misra and Khurana, 2008). MetS is defined as a co-occurrence of medical disorders consists of elevated blood pressure, elevated fasting sugar level, high serum lipid profiles and abdominal obesity (excess body fat around the waist) (Alberti and Zimmet, 1998, Zimmet et al., 2005). The effects of helminthes on type 1 and type 2 have rigorously been discussed in previous chapters. Intestinal helminths absorb lipids either from their host's gut or blood stream, which could reduce their host's circulating lipids and thereby minimize accumulation of plaques in vasculature (Hall et al., 2008). A study conducted by Wiria and colleagues describe that the interplay between helminth infection and carotid intima media thickness which is a marker for subclinical atherosclerosis was negatively associated (Wiria et al., 2013). This finding is supported by another study that *Opistorchis felineus* infection describing lower serum total cholesterol level and being a negative predictor of aortic atherosclerosis development (Magen et al., 2013). *O. felineus* is a close relation of *O. viverrini* which was the most frequent parasite in the Laos study.

Obesity is usually associated with chronic low-grade inflammation and alterations of immune cells composition in metabolic organs, especially in adipose tissues. Chronic low-grade inflammation associated with obesity contributes to insulin resistance and type 2 DM by inducing pro-inflammatory cytokines, chemokines, insulin resistance-associated adipokines and

acute inflammtory reactants are central to the MetS development (Wu et al., 2011). Helminth parasites are the strongest natural inducers of type 2 immune responses and counterbalance sterile metabolic inflammation and insulin resistance by re-shifting the balance from Th1 to Th2 and inhibiting of Treg cells and macrophage activation resulting in the protective alternative state from the maladaptive classical response (Odegaard and Chawla, 2011). Hussaarts and colleagues demonstrated in a study that chronic helminth infection reduced body weight gain by 62%, fat mass gain and adipocyte size by 89%, lowered whole-body insulin resistance by 23%, glucose intolerance by 16%; and improved peripheral glucose uptake by insulin sensitivity by 25% (Hussaarts et al., 2015). In spite of that, several modifiable risk factors such as advancing age with loss of muscle mass, physical inactivity, endocrine dysfunction, and genetic factors are also involved in the expression of syndrome. A number of circumferences such as endothelial activation and damage leading to changes in vascular tone, vascular reactivity, and coagulation and fibrinolysis pathways are contributing to pathophysiological mechanism of hypertension (Beevers et al., 2001). Insulin resistance causes endothelial dysfunction by decreasing Akt kinase activity, resulting in declined endothelium NOS phosphorylation activity (Muniyappa and Sowers, 2013). Visceral adiposity also fosters endothelia dysfunction through the effects of resistin, IL 6 and TNFα on endothelium NOS phosphorylation (Zhu et al., 2005). These interrelated factors share the common pathways to hypertension. However, the scientific linkage between helminth effect and hypertension is still undiscovered.

Deworming and helminthic therapy

The deworming removes the helminth infections and reduces the immune suppression of Th1, Th2 and Th17 and Tregs responses leading to the several inflammatory reactions. Therefore, administration of helminth related substance or therapy could restore the immune regulation mechanism of T cells and Treg cells which proves to have similar roles in chronic helminthic infections (Wammes et al., 2014). Helminthic therapy is currently being studied as a promising treatment for several autoimmune diseases including Crohn's disease (Hunter and McKay, 2004), asthma (Falcone and Pritchard, 2005) and ulcerative colitis (Saunders et al., 2007) using *Trichuris suis* ova. 72% of patients improved symptoms with no adverse reaction (Hunter and McKay, 2004). However, *T. suis* ova therapy showed no effect in allergic rhinitis, even though it induced a substantial clinical and immunologic response (Bager et al., 2012). Two previous studies reported that infection with hookworm (*N. americanus*) larvae was strongly

associated with protection against asthma symptoms (Scrivener S, 2001). It also led to a slight improvement in disease symptoms with Crohn's disease patients (Croese et al., 2006).While hookworms have some pathogenic potential effect (Hotez et al., 2004) they are likely to be safe at low infection intensities (Pritchard et al., 1999). However, no long-termed follow up has been assessed for clinical, parasitological and immunological consequences. Helminth derived molecules or products have also been used against allergic diseases, colitis and rheumatoid arthritis (Wammes et al., 2014). However, overall effects of deworming on autoimmune and inflammatory diseases in human studies have been inconsistent. In our study, we did not find any significant association of change in HbA1c level after deworming. This could be explained by the fact that significant clinical effects of deworming might take some time to establish. We could not follow up these clinical effects and we could not keep the participants free of reinfection during our study period.

Helminthic control strategy/Deworming program in South Africa and Laos PDR

Since recurrent helminth infections are very prevalent in the different provinces of Laos according to the existing helminth studies (Rim et al., 2003, Phommasack et al., 2008, Sayasone et al., 2011), the Ministry of Health and Ministry of Education collaborate on school deworming programs with health education, which takes place two times a year targeting schoolchildren. In addition, the Lao government has made considerable efforts to control STHs in women at reproductive age (Phommasack et al., 2008).

In South Africa, STH infection is most prevalent amongst socioeconomically disadvantaged children who live in densely populated and under serviced areas such as informal settlements. Studies reported infection rates from 20 to 90% among young learners. High levels of infection have been documented amongst children in all provinces, including KwaZulu-Natal, the Eastern Cape, Mpumalanga, the Western Cape and Gauteng (Ajoge et al., 2014). School-based deworming is universally accepted as a safe, simple and cost-effective measure. Even though the South African healthcare experts agreed that deworming is an important component of school health programme, the implementation of deworming programs in schools varies between regions. The departments of basic education and health have endorsed the regular deworming of learners in late 2015, and at a first stage, the government of South Africa's new national deworming program intended to deworm 7 million primary school children in 2016 to improve children's health. The goal is to reach a minimum target of regular administration of

deworming medication to at least 75% of school-age children in general and up to 100% of those at risk.

Deworming becomes a key element to fight the helminthiasis from a global perspective. Mass drug administration (MDA) programs in endemic countries usually involve cooperation between ministry of health, and pharmaceutical companies, or international donors/organizations. MDA programs are mainly targeted at preschool- and school-aged children. Therefore, children who do not attend school are missed (Barry et al., 2013). The major barriers to cost-effective helminth control are the lack of research evidence on the geographical distribution of infection and the occurrence of co-infections or re-infection (Prichard et al., 2012). The success of MDA also depends on drug coverage as well as compliance of the community. It is also important that the community in endemic countries would need to have the knowledge about the nature of helminthes, their modes of transmission, the helminth associated diseases, the consequences of helminthiasis and the benefits of MDA. A mixed method survey conducted by Phongluxa and colleges revealed that the concept and practice of MDA was well accepted in affected Laos communities and the main reason was to avoid severe complication of schistosomiasis (Phongluxa et al., 2015). However, a systematic review revealed that deworming is unlikely to improve overall public health. In addition, global deworming program could not kill a variety of different helminth infections as different helminthes infections require different treatment regimes, therefore, global drug coverage for different helminth infections could fall below target levels and infection levels remain the same or even high (Allen and Parker, 2016).

Diabetes mellitus or chronic diseases prevention program in Laos PDR and South Africa

Starting from 2014, the Laotian government engaged the DM related activities and projects to improve the diabetic health care in the country. Since there is no DM national policy, the ministry of Health and international non-governmental organizations have collaborated and developed 3 years project plan (2017-2020) which is funded by International Diabetes Federation, to tackle DM issues and its related co-morbidities. The project includes training the health care providers in targeted tertiary hospitals in four provinces (Vientiane Capital, Champasak, Luang Prabang and Savannakhet) to provide a standardized care and managmenet of DM as well as hypertension. Currently, more than 12,000 DM patients are receiving a proper treatment in the hospitals (WDF, 2017).

Type 2 DM ranks fourth in South African ambulatory primary care setting with 65% of the adult population are affected. However, recent studies reported it could be as high as 33% in some communities of Cape Town (Mash et al., 2015). According to our HbA1c assessments in South African study, most of the children fell into preDM group, which means a high risk of having DM in near future. The IDF & International Society for Pediatric and Adolescent Diabetes has developed the guideline for Diabetes in Childhood and Adolescence to assist health providers and caregivers in managing diabetic children and adolescents in a standardized way. The Kids and Diabetes in Schools (KiDS) project is designed to support the rights of children with diabetes, for happy school days in increasing the awareness of the needs of diabetic children (IDF, 2015). To minimize the burden of NCDs in South Africa, the department of health set out a detailed plan in the 2011 South African Summit on prevention and Control of NCDs with objectives, indicators, activities and time frames. Increasing the percentage of persons controlled for hypertension, DM and asthma by 30% are among 10 goals with specific targets is missioned to be achieved by 2020. Other target programs are in reducing the relative premature mortality by at least 25%; reducing tobacco use by 20%; reducing the per capita consumption of alcohol by 20%; reducing mean population intake of salt <5 grams per day; reducing the prevalence of people with raised blood pressure by 20%; increasing the prevalence of physical activity (defined as 150 minutes of moderate-intensity physical activity per week, or equivalent) by 10%; reducing the prevalence of cervical cancer and every woman with STDs to be screened every 5 years; and increasing the number of people screened and treated for mental disorder by 30%. More investment in innovation, scientific enquiry, health systems reforms and legislative interventions are required to combat NCDs. A healthy lifestyle is an obvious key to the prevention of approximately 80% of NCDs. To improve health systems to attain higher levels to control for hypertension, DM and asthma, integrated chronic care model with training of community health workers in adherence of counseling, monitoring, diagnoses of high CVD risk people and referring people suffering from NCDs has been implemented in 3 sub districts of South Africa in 2013. This chronic care model has been expanding into 10 full districts in 2015 and the model will then be evaluated in terms of the feasibility and integration for further application.

Limitations of our studies

Re-infections, co-infections and multiparatism have been major limitations in our studies. Moreover, we did not include any immunological measures in our studies; therefore. Therefore,

we were not able to further explore the exact patho-physiological mechanism associated with the dual diseases burden. We expect that different parasitic species could have different immunological reactions in the host and they lead to remarkable impact in HbA1c levels (e.g hookworm used to act in a different way compared to other STH). In LADUBU study, a causal link could not be identified as the study was cross-sectional in nature. In South African study, we have seen the HbA1c level has been increased after deworming. However, we could not assume the causation as the association is not statistically significant. We believe that there were a number of factors influencing the disease interaction between infections and HbA1c levels. Some medical diseases related to abnormal haemoglobinopathies such as thalassemia, sickle cell anemia might have impact on the HbA1c assessment and other blood test of haemoglobinopathy for the medical related diseases, however, the assessment of anemia with the HemoCue Hb 301 system was considered in our study.

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Chapter 9

Conclusion and recommendations

9.1 Conclusion and recommendation for DBD

In this PhD thesis, we looked into ways how different diseases interact with each other, particularly on the association of DM with NTD. DM is well known for its link to altered immune responses and immunological complications and the drastic rise of DM in LMIC places it as a central contributor to the DBD in a large part of the world. Dengue viral infection and helminthic infections represent an important example of the overlap between NTDs and NCDs, especially among the poor society. However, it is also urgently required to have a better understanding of co-morbid pathogenesis and to identify other NCDs interactions among the NTDs or other NTDs interactions with DM. The findings of the study act as an eye opener for health policy makers and also draw an attention from the health researchers as there are relatively few literatures about this relationship. A key factor is the development of dengue vaccines or helminthic therapy, which could be prioritized for individuals with underlying DM, depending though on the direction of the association between specific infections and DM. The adoption of the strategy addressing both diseases which is a crucial first step should be followed by the implementation at the country level. A policy with multi-sectorial approach involving all stakeholders; public health decision makers, healthcare providers, non-governmental organizations, research institutions, community and individuals should be necessitated.

9.2 Conclusion and recommendation for DM and Dengue viral infections association

9.2.1 Conclusion

The literature review and epidemiological studies looking into the dual burden of DM and dengue viral infections are still scarce due to low political interest to prioritize the research attention and also lack of coordinated efforts between endemic countries, despite the burden of these diseases being enormous from social and economic point of view. Large dengue outbreaks have been occurred worldwide in recent years. The objectives of the global strategy in reducing the burden of dengue by WHO are to reduce dengue mortality by at least 50% by 2020 and to

reduce dengue morbidity by at least 25% by 2020. By addressing the following recommendation, the goal of global strategy could be achievable in near future and the DM patients with dengue infection would also have a better prevention and treatment.

9.2.2 Recommendations

- Timely dengue case diagnosis and appropriate clinical management can prevent the dengue related mortality. Additional clinical studies to develop a standardized clinical guideline and treatment protocol for the dengue infected DM patients to prevent a severe dengue are suggested.
- As most of the previously conducted studies were case-control studies, prospective studies in high endemic area of dengue viral infection which explain the relevance of any NCDs comorbidity with dengue infections are needed.
- Glycemic assessment in patients with suspicion of dengue viral infections or assessment of dengue viral infection along with other arbovirus differential diagnosis such as Virus Zika or Chikungunya should be performed among DM patients to prevent a severe form of these infections.
- Future studies in different countries settings should be conducted with similar epidemiological methods and outcome measures, to improve the comparability of the research in the context of DM and dengue viral infection.
- Studies focusing on detailed immunological and pathophysiological mechanism on association between DM and dengue viral infection are also pivotal in better understanding of comorbidity.
- Effective vector control measures are critical to achieving and sustaining reduction of dengue morbidity. Therefore, it should also remain as a pillar for dengue prevention and control.
- Risk assessment, integrated epidemiological and entomological surveillance and improved reporting of dengue cases at various levels of health care management are also critical component in dengue control and measures. It also relates to in preventing of severe dengue cases among normal individuals as well as DM patients.

- More accurate data on diseases burden of dengue and DM data are recommended for research prioritization, health policy, and resources management toward reducing this poorly controlled disease.
- Additionally, clinical trials with dengue vaccination should be considered for all age groups and the current dengue vaccination implementation should be expanded to all endemic countries and also including DM patients and patients with other NCDs.

To translate these recommendations into action, advocacy and resource mobilization, partnership, coordination and collaboration, capacity building, monitoring and evaluation are essential for effective implementation as the global strategy.

9.3 Conclusion and recommendation for DM and parasite infections association

9.3.1 Conclusion

The research on co-morbid conditions and their influence on the progression of another disease are very insightful and useful in clinical setting of disease management. Reducing case morbidity or mortality through management of associated risk factors can definitely improve clinical outcomes. Through actual understanding of the complex interactions between or parasite infections on co-morbid DM condition, we will be able to determine in prioritizing target populations for prevention, interventions and effective resources allocation for treatment and care of comorbid patients. Our findings contribute to the steps in developing potential novel therapeutic strategies (either a vaccine or a drug) aimed at preventing and/or delaying the disease onset and/or decreasing the severity of DM. A series of evidence based recommendations are also available to health authorities especially in helminth and parasite endemic countries. Furthermore, the findings from this study provide a basis to conduct other comprehensive studies, investigating the impact of antihelminth treatment on the immune system and on insulin resistance. Such studies need to take different helminth species into consideration, given the potentially different effects.

9.3.2 Recommendations

- As taeniasis is the immediate source of cysticercosis in pigs and humans, addressing taeniasis issue with estimating of the prevalence or incidence requires large-scale

collection and screening of stool samples and it could be a challenge in a taeniasis elimination program.

- It is evident that future *Taenia* research in endemic countries still needs the use of more robust and reliable diagnostic protocols for the animal and human studies.
- The valid epidemiological data of taeniasis in human as well as affected animals is still inadequate; therefore a basic research and appropriate surveillance system of taenia infection in endemic regions should be addressed to identify high risk population and to focus prevention and control measures in those areas.
- If there is evidence of taeniasis infection, infected individuals and animals should be properly treated and health awareness of community to undercooked meat should be promoted to avoid reinfections.
- Longitudinal and mechanistic studies need to evaluate how Taenia infections and DM influence each other in order to determine if that relationship has clinical relevance.
- According to our study findings, further interventional studies are urgently needed to evaluate the long-term benefit of *H. pylori* eradication for prevention and progression of DM.
- Due to the evidence of *H. pylori* role on blood sugar concentration, preventive measures, such as increased hygienic conditions and treatments using combination of antibiotics and proton pump inhibitor, should be targeted to high risk communities.
- As a couple of studies including our study have remarked that the causal link of these relationships have never been clearly identified, well-designed household cluster randomized control trial to assess the effect of anthelminthic treatment on glucose metabolism with clean and undetectable worm status will be needed to avoid the reinfection of treated individuals.
- However, reinfection must also be addressed with some basic helminthiasis research initiatives in affected society.
- Given evidence of antibiotic resistance to H. pylori infection, choosing the right drugs (antibiotics) for DM or high risk population becomes a necessity to eradicate the infection.
- Like other infectious diseases, as an alternative treatment to deworming or antibiotics, developing a potential vaccine therapy which doesn't currently exist for taeniasis or *H.*

pylori infections, could be an interesting and cost-effective strategy for sustained control and treatment of infections.

- More accurate epidemiological and surveillance data will be needed to prioritize this dual burden of diseases in healthcare planning of resource allocation, and investing of new diagnostic techniques, development of new therapeutic helminthic or parasitic related compound or vaccine which are expected to treat also DM patients in coming decades.
- In the context of assumed protective effect of some helminthes on DM, well-designed clinical trials with large scale deworming would be needed to assess the advantages and disadvantages of deworming in the population.
- In regards to potential helminthic therapy for DM, proper trials with adequate sample size, dose of helminthic therapy used, and longer follow up period to monitor and evaluate the clinical, parasitological and immunological features of the participants should be considered.