Helminth infection and metabolic disease: *Strongyloides stercoralis* infection and type 2 diabetes mellitus in an Aboriginal community

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Statement of the Contribution of Others

Nature of Assistance	Contribution	Names
Supervision	Preparation and review of proposals Collaboration in writing of all papers	Professor Robyn McDermott Professor Adrian Esterman
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Abstract

Recent years have seen an upsurge in interest in the relationship between helminth infections and metabolic diseases such as insulin resistance and type 2 diabetes mellitus (T2DM). Limited clinical and laboratory studies have suggested that chronic helminth infections may protect against the development of T2DM, and have suggested an immunometabolic mechanism for such an effect.

Aims

This thesis seeks to examine the relationship between *Strongyloides stercoralis* infection and T2DM in an Australian Aboriginal community, and to examine the importance of this relationship in the context of efforts to treat and control this infection.

Methods

The thesis reports on a cross sectional observational study of adults attending the health centres in an Aboriginal community, testing them for both strongyloides infection and T2DM, and establishing the nature of the relationship between these two conditions. A cohort of infected and un-infected subjects is thereby established and followed over a three-year period, enabling characterization of both the treatment outcomes for this infection at 6 months and three years in the context of T2DM, and the effects that treatment for strongyloides has on subsequent metabolic parameters. In addition, the relationship between *S. stercoralis* infection, T2DM and eosinophilia is examined, and limited studies into the cytokine responses in treated individuals are performed

Results

The study demonstrates a strong negative association between pre-existing *S. stercoralis* infection and T2DM. It shows T2DM is a predictor of treatment failure for *S. stercoralis* at 6 months, and demonstrates that eosinophilia is not a reliable predictor of S stercoralis infection in this community, but is a more constant finding in those patients with *S. stercoralis* and T2DM. The three-year follow up shows that ivermectin is an extremely effective treatment for *S. stercoralis* infection in this community, and demonstrates that treatment of *S. stercoralis* infection is associated with an increased risk of developing T2DM and impaired glucose tolerance when compared to an uninfected and untreated group.

Conclusions

The thesis provides evidence to support a protective effect for *S. stercoralis* infection against T2DM and suggests an immunometabolic model to explain the negative association which has been found. These findings are discussed in the context of ongoing efforts to control and eliminate strongyloides infection in Australian Aboriginal communities.

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Chapter 1 Helminth Infection and Metabolic Disease in Humans: an Introduction and Literature Review

1.1 Helminth Infection and Type 2 Diabetes Mellitus

Recent years have seen an upsurge in interest in the relationship between helminth infections and metabolic diseases such as insulin resistance and type 2 diabetes mellitus (T2DM) - two areas of medicine that may have previously seemed distant from each other both in geographical and pathophysiological terms. Whilst both conditions are common in a global sense, their regions of greatest prevalence have traditionally had little overlap, with helminth infections common in the developing world, and T2DM most prevalent in the developed world. Past decades have however, seen an epidemic of metabolic disease and T2DM in "transitional" societies (those undergoing the change from "developing" to "developed") where helminth infections have been common in the past.

The "hygiene hypothesis", current since the 1980's, has postulated that the relatively low levels of allergic and autoimmune diseases seen in some developing societies may reflect the high levels of infectious diseases there, through modulation of the immune system by early exposure to these conditions[1, 2]. The apparent inverse relationship between parasitic diseases such as helminth infections, and metabolic disease can therefore be seen by analogy as an extension of this theory.

The world is currently experiencing an epidemic of Type 2 diabetes mellitus and its consequences. It is estimated that the total number of people living with T2DM will exceed 366 million by 2030[3]. This is occurring not just in the developed world, but also to an increasingly greater extent in the developing world, where it was until recently an uncommon condition. In China for example, the national prevalence of T2DM was 11.6% in 2010 compared with 5% in 2001 and 1% in 1980[3]. Diabetes in the developing world is more commonly poorly controlled and it is being observed in progressively younger populations. It is more likely to remain undiagnosed, or to be treated inadequately. This is also the case for relatively underprivileged populations living within developed societies, in particular the indigenous populations in North America and Australia[4].

In addition to T2DM, the antecedent conditions that constitute the metabolic syndrome, such as obesity, dyslipidemia, hypertension and insulin resistance, are also increasing in

prevalence. It is a commonplace to attribute these increases to the adoption of "Western" diets with the effects of excessive nutrition and high fat diets being well documented. However, changes in diet are not the only demographic and environmental changes observed in such transitional societies, with increasing urbanization, changes in patterns of physical activity, standards of hygiene and patterns of infectious diseases all being evident. It is therefore plausible that these factors could also influence the prevalence of T2DM.

An improved understanding of the immunology underlying helminth infections and their ability to modulate the human immune system, and an improved understanding of the relationship of T2DM and metabolic disease to inflammation have prompted further interest in this relationship.

Helminths are known to be master manipulators of the host immune system, capable of inducing modified T helper cell type 2 (Th2) weighted immune responses which have the dual effect of reducing harmful inflammation in the host, while ensuring the parasites' survival[5]. At the same time, T2DM is increasingly seen as a disease of chronic inflammation, with inflammation in adipose tissue in particular both a cause and consequence of insulin resistance in peripheral tissues, and ultimately T2DM and its metabolic consequences[6, 7]. Seminal research conducted in the early years of this decade has established a link between helminth infections, subsequent eosinophilia and Th2 weighted immune responses, and the molecular pathways that regulate glucose metabolism and insulin sensitivity[8, 9]. This laboratory work preceded clinical and observational studies in this field and had the effect of prompting a number of studies, including the current one, into this phenomenon.

Questions regarding the relationship between helminth infection and metabolic disease have far more interest than matters of academic interest, and clearly may be of importance in decisions of a public health nature in both developing and transitional societies. In addition to the decline in helminth infections that is an inevitable consequence of improved levels of hygiene and housing, there are well-developed WHO public health programs that aim to reduce and eliminate these conditions worldwide. Examples include programs to eradicate onchocerciasis, lymphatic filariasis and schistosomiasis, which have met with considerable success in reducing the public health impact of these damaging conditions. WHO sponsored programs to reduce the impact of soil-transmitted helminth (STH) infections have met with more questionable success, and the health benefits of such programs have been brought into question[10, 11]. Should the link between metabolic disease and helminth infection be more

firmly established, then these public health interventions and their consequences clearly face further questions in the context of societies that already face an epidemic of metabolic and non-communicable diseases in the coming decades.

1.1.1 *Strongyloides stercoralis*

Strongyloides stercoralis is a soil transmitted helminth (STH) infection affecting mostly poor societies in tropical and temperate climates worldwide, and is recognized as one of the WHO neglected tropical diseases. Estimates of its prevalence have depended on mainly incomplete epidemiological data, and have usually been stated as 70 to 100 million cases. More recent estimates have revised this up to 370 million[12]. The infection was first identified in the 1800s and much work on its life cycle and transmission was completed in the early 20th century[13]. As hygiene and living conditions improved in the developed world through the second half of the 20th century, the infection became increasingly uncommon, and it is now rare in developed societies. It remains common in developing and transitional societies, and in certain under-privileged groups living within developed societies[14].

1.1.1.1 Lifecycle

The widespread distribution of this infection, and the nature of its transmission and pathophysiology make it an ideal candidate to study in the context of metabolic disease. To understand this, it is necessary to understand some key points regarding the life cycle, transmission and clinical effects of the infection, as these are central to understanding its ability to produce chronic disease[13, 15]. As with hookworm species, infection occurs when the infective larvae, present in faeces or in contaminated soil, penetrate the host's skin. The larvae migrate through the bloodstream and body fluids of the host to the lungs, where a series of developmental stages occur before the larvae finally migrate to the intestine of the host, and adult female worms are established in the sub-mucosal layer.

These female worms produce eggs by parthenogenesis, which then hatch into larvae whilst in the intestine. "Rhabditiform" larvae are passed in the faeces and one of two external life cycles is completed. The rhabditiform larvae can complete a number of moltings to produce infective, filariform (L3) larvae and then re-infect a new host. Alternately these larvae may develop into externally living adult male and female pairs, which then produce larvae by sexual reproduction. The disposition of rhabditiform larvae to one of these two outcomes is incompletely understood and is thought to depend upon both internal factors, such as the host's immune response, and external factors such as temperature and moisture[16].

A crucial point of difference in the lifecycle of *S. stercoralis* when compared to other STH is that rhabditiform larvae can complete the transition to infective larvae whilst within the host's intestine, and these "auto-infective" larvae can then penetrate the bowel wall, or the skin of the anal margin, thus completing an internal re-infection cycle. In this way *S. stercoralis* differs from all other STH infections in that chronic infections can be established in the absence of ongoing environmental exposure to the larvae. This enables the establishment of "life-long" systemic infections both in individuals living in endemic communities, and in those who have migrated from endemic to non-endemic regions. The continuing presence of the worm in the superficial layers of the intestinal wall, and the intermittent penetration of the bowel wall by larvae which then migrate through the hosts body, will also presumably provide a more constant and sustained stimulus to the host's immune system than an infection which is confined to the lumen of the bowel and where the life cycle of the worm relies on entirely external mechanisms and repeated re-exposure to the eggs or larvae, as is the case in other STH infections.

The worm's ability to sustain chronic infections in its host also means that human beings are the likely principal reservoir of infection in endemic societies, although recent work also suggests that dogs may constitute an additional reservoir of infection[17]. Infective L3 larvae produced via parthenogenesis are thought to survive only about 14 days in the external environment, presumably under favorable conditions[18]. The L3 larvae that arise from the external sexual reproductive cycle may survive for longer periods, perhaps even indefinitely, but there is only ever one generation of free living adult worms, and the relative contribution of the external and internal reproductive cycles is likely to depend on numerous external and host factors[16]. The location of the reservoir of infection is of importance from a practical point of view. If the reservoir is principally, or effectively entirely within the human population, then the infection lends itself to elimination via pharmacological means such as "case finding and treatment" and/or mass drug administration (MDA). If the environment constitutes a significant reservoir, then environmental manipulation will be crucial to the control of the infection.

1.1.1.2 A "neglected" neglected tropical disease

Further reasons for the relative neglect of strongyloides infection stem from peculiarities in the symptoms and disease burden produced by *S. stercoralis* infection, and the means available for diagnosis and treatment[14].

While *S. stercoralis* is a common infection worldwide, the burden of disease that it produces remains less certain. Early descriptions of the disease describe it as a cause of diarrhea and gastrointestinal symptoms[13]. The classic description of the disease in western society was in returned prisoners of war, where high rates of itchiness, rash and gastrointestinal symptoms were reported[19, 20]. This included the pathognomonic creeping eruption "Larvae currens" produced by migration of larvae through the host's skin.

In practice, these manifestations seem to be less common in endemic societies where the presence of more longstanding infections may result in fewer symptoms[15], and where conceivably, the mild or irritating symptoms produced are obscured by the more pressing presence of other infectious diseases. A study conducted in Africa described higher incidences of self-reported "general ill health", gastrointestinal disturbances and cough with wheeze, in subjects with *S. stercoralis*, but still the majority of those infected reported no symptoms[21]. The absence of association with more clinically damaging conditions seen in other STH infections, such as maternal and childhood anaemia and failure to thrive, has led to the relative neglect of *S. stercoralis.*

The principal clinical complication of *S. stercoralis* infection in developed societies is that of hyper-infection syndrome[22]. Hyper-infection occurs most frequently in circumstances where the host's immunity becomes impaired in the presence of an acute or chronic *S. stercoralis* infection. The adult worms in the bowel begin to produce auto-infective larvae in huge numbers and these penetrate the host's body, carrying with them gram negative and other bacteria, and resulting in widespread infections and septicemia. Established cases are often misdiagnosed, are difficult to treat, and have a high mortality rate. This syndrome is most commonly a consequence of the administration of steroids or other immunosuppressant drugs, or in co-infections with the retrovirus HTLV-1, but has also been reported as a complication of HIV infection, alcoholism, haematological and solid malignancy, and a variety of other conditions. Infrequently it may occur in the absence of any known precipitating factor. Although it undoubtedly occurs in developing societies, it most likely often goes unrecognized, and its iatrogenic causes are more frequently encountered in the developed world. It is therefore a significant risk for those migrating from endemic to non-endemic societies, or those living in at-risk populations within developed societies.

Difficulties with the diagnosis of *S. stercoralis* have in the past lead to underestimates of its prevalence, and contributed to its relative neglect in comparison to other STH infections[23].

S. stercoralis eggs are not passed in the faeces and so conventional and relatively simple parasitological investigations used for the detection of other STH infections, such as the Kato-Katz method are unlikely to detect the infection. Specialized techniques for detecting the larvae such as the Baermann funnel method, the Harada-Mori filter paper system, and Kogar agar plate culture have been developed, and have improved sensitivity[24]. These methods are however, time and labor intensive, rely on the collection of large and multiple faecal specimens, and are dependent on experienced and readily available laboratory staff. In practice, this makes such investigation difficult, and often impractical in endemic societies. While the tests are specific in experienced hands, their sensitivity is low even under ideal circumstances.

The poor performance of direct methods of diagnosis has led to the development of various other means of diagnosis for *S. stercoralis*, including immunological tests and molecular diagnosis, however, questions remain over the sensitivity and specificity of these methods. Determination of sensitivity and specificity of indirect diagnostic tests is rendered more difficult by the absence of a "gold standard" diagnostic test with which to compare. Recent studies have looked at the performance of indirect tests in the diagnosis, and follow up of *S. stercoralis* and have included commercially produced ELISA tests detecting IgG antibodies[25, 26]. They have concluded that these tests are sensitive and specific enough to be of use in the diagnosis and follow up of treatment for *S. stercoralis* infection, however, questions still remain over the cut-off point used for diagnosis of infection in endemic societies, and the definition of treatment success in relation to antibody changes after treatment.

Other advances in diagnosis include the development of faecal PCR tests for the detection of worm DNA in faeces. These tests promise improved sensitivity and are less "operator dependent", but still rely upon the presence of larval shedding in the faeces, which is known to be only intermittent and light in chronic infections[24]. A recent study suggested that faecal PCR was superior to direct examination in its sensitivity, but that both tests, either alone or in combination, were still less sensitive than serology[27]. Clearly the development of a rapid, on site detection method with adequate sensitivity and specificity would contribute to the understanding and treatment of this infection.

Treatment for *S. stercoralis* also differs from the treatment for other STH infections, and has thus contributed to its exclusion when consideration is given to worm control programs[23].

Albendazole, which is the standard treatment for all other STH infections, is known to be relatively ineffective against *S. stercoralis*, even when given in relatively prolonged courses[2, 28]. Ivermectin is now considered to be the treatment of choice, although questions still exist with regard to the best dosing regimen. Other anthelminthic drugs such as thiabendazole have been shown to be equally effective, but have a higher side effect profile[29]. Recently another drug from the avermectin group, moxidectin, has been shown to be effective[30].

1.1.1.3 Strongyloides infection in Australia

The situation with regard to strongyloides infection in Australia to some extent reflects that which exists in the wider world. The overall prevalence of *S. stercoralis* infection in non-Aboriginal Australia is low, in keeping with its status as a developed country, with the exception of some immigrant and refugee populations originating from endemic regions. This is not the case however, in Aboriginal communities in the north of the country where health and hygiene conditions more closely resemble those seen in transitional and developing societies. Although epidemiological data are incomplete, prevalence of up to 60% has been reported in some studies[31], and attempts are currently underway to improve upon the quality and range of data available, raise awareness of the condition and systematize the investigation and treatment of those in affected communities[32]. The health impact of *S. stercoralis* infection is largely unknown, although hyper-infection and severe strongyloidiasis is a recognized problem in central Australia, and may be compounded by the presence of HTLV-1 infection, which is a known predisposing factor for this condition[33]. Data are lacking on the effectiveness of ivermectin treatment in the setting of Aboriginal communities, and on the effectiveness of attempts to control or eliminate the infection through pharmacological means[34].

1.1.2 *Type 2 diabetes mellitus and inflammation*

The link between obesity, the metabolic syndrome, T2DM and inflammation is now well established. As the role of inflammation and the immune response in T2DM and insulin resistance is central to this thesis it is necessary to review the main body of knowledge in this regard.

The first recognition of a role for inflammation in obesity and insulin resistance came 25 years ago with the discovery that levels of the pro-inflammatory cytokine TNF-α were elevated in the adipose tissue of obese individuals[35]. Since that time, the role of adipose tissue as an endocrine organ has been revealed, with its production of numerous pro and

anti-inflammatory cytokines such as IL-1, IL-6, TNF-α and Il-10, along with adipokines such as leptin and adiponectin. Subsequently it was revealed that, although adipocytes are responsible for the secretion of some of these cytokines, tissue macrophages within adipose tissue constitute the major source of cytokine production[36].

The adipose tissue of obese individuals is known to be an inflammatory environment, with necrosis of adipocytes attracting type 1 or "classically activated" macrophages, associated with the production of pro-inflammatory cytokines. This necrosis and inflammation is thought to be attributable to excessive nutrition and rapid expansion of adipose tissue, resulting in relative hypoxia and poor blood flow and adipocyte death, as well as the direct effects of high levels of free fatty acids. By contrast, the adipose tissue of lean individuals is a relatively anti-inflammatory environment with type 2 or "alternatively activated "macrophages (AAM) distributed throughout the tissue, responsible for the production of anti-inflammatory cytokines such as IL-10.

The presence of either type 1 or type 2 macrophages, and the cytokines they produce then has direct consequences for glucose metabolism and insulin sensitivity, with pro-inflammatory cytokines reducing the sensitivity to insulin of adipose tissue and other effector organs such as liver and skeletal muscle, while anti-inflammatory cytokines have the opposite effects.

Recruitment of alternatively activated macrophages to adipose tissue is central to maintaining energy homeostasis and occurs via an IL-4 dependent pathway. AAMs not only produce anti-inflammatory cytokines such as IL-10, but they act to down-regulate classically activated macrophages, and are necessary for the development and sustaining of healthy adipose tissue. In addition, cells of the adaptive immune system are involved in the ongoing maintenance of an anti-inflammatory environment, with a reduction in the number of Th1 weighted T cells, and an increase in the proportion of T reg cells[9].

Recent work has concluded that eosinophilic infiltration of adipose tissue is the strongest stimulus for producing and sustaining AAMs in these tissues. This was supported by the establishment of experimental helminth infections in mice producing tissue eosinophilia that then sustained AAMs in adipose tissue, and produced an improvement in insulin sensitivity[8].

It is possible therefore to propose a model based on adaptions to survive different infections that could connect the immunological states in individuals, and their differing effects on glucose metabolism[9]. Acute bacterial and viral infections, which pose an immediate

existential threat to the organism, are met with a prompt and often massive immune response, with mobilization of large populations of cells from the innate and adaptive immune systems. This is associated with a Th1 weighted immune reaction, involving the production of pro-inflammatory cytokines such as TNF-α, IL-6 and Il-1B, and these cytokines have the effect of reducing insulin sensitivity, impairing the peripheral storage of nutrients and promoting gluconeogenesis in the liver, thereby ensuring the ready availability of substrate for the immune cells produced in response to infection. Thus, reduced insulin sensitivity and relative hyperglycemia is an adaptive response to enhance survival during acute life-threatening infections.

Conversely, infections with parasites rarely constitute a sudden existential threat to the host. Rather, the threat to the host comes more through a long-term impost on nutritional resources, and potentially, the effects of excessive and prolonged inflammatory reactions in response to the continuing presence of parasite antigens. Parasite infections are associated with Th2 weighted immune responses, with the production of Il-4 and IL-13, recruitment of eosinophils and AAMs to affected tissues and the production of anti-inflammatory cytokines such as IL-10 and IL-1RA. These have the effect of increasing insulin sensitivity in peripheral tissues, which enhances the storage and efficient use of nutrients, and has the dual effect of reducing the availability of nutrients for the pathogen, thereby reducing its potential for survival and growth, while conserving resources for the host and thereby improving its chances of survival in the face of long-term infection. The damping down of the host inflammatory response also has the effect of reducing the potential damage inflicted on host tissues by longstanding inflammation[9].

1.1.3 *A model for the immune-metabolic effect of infection*

It is not difficult therefore to conceive of a circumstance where the dysregulation of the inflammatory response to recurrent bacterial and viral infections could lead to a prolonged reduction in insulin sensitivity, hyperglycaemia and subsequent T2DM. Similarly, prolonged exposure to parasitic infections could result in a persistent modified Th2 weighted inflammatory environment, with recruitment of AAM's to adipose tissue, and subsequent enhanced insulin sensitivity and a reduced incidence of T2DM.

For subjects living in transitional societies, such as the Aboriginal people who are the subject of this thesis, there may therefore be more than one pathway to insulin resistance and T2DM. In Australian Aboriginal populations, there is clear evidence for increasing rates of obesity

and T2DM over a period of decades[37, 38]. While the developed world has seen the simultaneous advent of over-nutrition and the reduction of infections of all kinds through improved hygiene, only a part of this transition has been achieved in Aboriginal societies. Food security, and indeed over-nutrition may have generally become more established, but infections of all kinds, particularly bacterial skin infection, chronic otitis media, recurrent upper and lower respiratory tract infection, bronchiectasis, and periodontal and dental disease are all still extremely frequent in these societies. On the other hand, efforts to control and eliminate some parasitic infections have met with greater success, with improvements in housing and the widespread use of albendazole in public health programs leading to a marked and progressive reduction in the prevalence of hookworm infection in the same communities[39, 40]. The relative roles of inflammation from infectious causes, and inflammation from the effects of obesity and over nutrition, and the mitigating effects of parasitic infection in the pathogenesis of T2DM in these populations, is a topic that has received little attention. Clearly, understanding the complex of factors in play may be of importance to the means employed to both treat and prevent T2DM in these communities.

Alternate explanations may also be proposed to explain the prevalence of diabetes in Aboriginal populations. The role of the gut biota in metabolic disease is being seen as increasingly important. Subjects with T2DM are known to sustain a different gut flora to non-diabetics[41] and this may responsible for maintaining a more pro-inflammatory environment within the body generally. Repeated doses of antibiotics in response to recurrent bacterial infections that characterize the early life of many indigenous children, could lead to a permanent change in the gut flora. Furthermore it is known that the presence of helminth infection itself can affect the gut biota[42].

Genetic predispositions to T2DM, such as the role played by genetic polymorphisms in the expression of cytokines, clearly may also be of importance in Aboriginal populations. For example, studies in the investigation of Sudden Infant Death syndrome seem to indicate that Aboriginal populations may carry genes expressing lower levels of IL-10[43] and higher levels of pro-inflammatory cytokines, but this has not been investigated in the context of T2DM.

1.2 Clinical Studies into the Relationship between T2DM, Metabolic Disease and Helminth Infections- a Review of the Literature

Over the past 7 years a number of clinical studies have appeared which examine the relationship between T2DM and helminth infections. These studies have examined helminth infections both as an outcome, and as a determinant of T2DM. In addition, one prospective trial of treatment for helminth infection and its effect on glucose metabolism was conducted and reported on during the course of this thesis project[44]. A number of studies have looked at helminth infections in relation to other complications of the metabolic syndrome such as atherosclerosis, atherogenic index and cardiovascular disease. Following on from these studies, there has been one meta-analysis examining the relationship between T2DM and helminth infection, and numerous review articles examining these topics.

1.2.1 *Search strategy*

The strategy adopted for this review employed a search of MEDLINE and EMBASE data bases. It was restricted to human studies with abstracts in English, and searched for research articles, review articles, meta-analyses and systematic reviews, conference abstracts, editorials and letters. The papers which constitute the subject matter of this thesis were not included. 768 abstracts were retrieved using this search strategy, and the titles were manually scanned for relevance. A total of 67 studies were identified and had their abstracts reviewed. The references in all review articles were also examined for relevant papers. This resulted in the inclusion of a total of 38 publications (15 research articles, 18 review articles, 1 meta-analysis and 4 others). The search strategy employed and a full list of the references identified is presented in Appendix 1.

1.2.2 *Helminth infection and T2DM*

To date, cross sectional observational studies that examine the relationship between helminth infection and T2DM and the metabolic syndrome, have found a negative association. The first study to report upon a relationship between diabetes and helminth infection was an observational study from Turkey[45]. The investigators here were concerned with soil transmitted helminth infection as an outcome and included patients with both T1DM and T2DM in the survey. To the author's surprise they discovered a negative association, with 47% of diabetic patients testing positive, compared to 55% of 1024 controls ($p<0.05$). They explained the reduced prevalence by the increased surveillance that diabetic patients receive

from their primary care practitioners, and did not consider the possibility of a protective effect from helminth infection.

The first such study to consider this possibility appeared in 2010, although the authors again designed the study with diabetes as the exposure and filarial infection as the outcome. Aravindhan et. al examined the prevalence of lymphatic filariasis (LF) among diabetic subjects, as part of the CURES (Chennai Urban Rural Epidemiology Study) in southern India[46]. 1416 subjects were tested, and statistically significant lower rates of LF sero-positivity were found in those with existing diabetes (4.3%) and newly diagnosed diabetes (5.7%), when compared to those with pre-diabetes (9.1%) and non-diabetics (10.4%). While the relationship held when adjusted for age, sex and socio-economic status, no adjustment was made for differences in BMI, even though BMI was significantly higher in diabetic groups compared to non-diabetic. This study also examined the level of circulating filarial antigen, which was lower among diabetics, and the levels of selected pro-inflammatory and anti-inflammatory cytokines. Those with positive filarial antigen had lower levels of the pro-inflammatory cytokines TNF-α, IL-6, and GM-CSF. No difference was present in the levels of anti-inflammatory cytokines. The authors suggest that the observed lower incidence of T2DM could be related to an amelioration of the pro-inflammatory environment associated with T2DM.

A study published in 2013 reported on the relationship between past schistosomiasis and T2DM in a rural Chinese population[47]. A self-reported history of schistosomiasis, validated by government records, and a number parameters measuring T2DM and insulin resistance were measured in 3913 subjects over the age of 60. There was a negative association between past infection and all the metabolic parameters measured, and this relationship persisted when adjusted for numerous demographic and anthropometric factors such as sex, age, BMI and diet. Another study conducted in China looked at past schistosome infection and the components of the metabolic syndrome, including central obesity, triglyceride levels, total cholesterol, HDL, LDL, plasma glucose levels and blood pressure, in a region where *S. japonicum* infection had historically been prevalent[48]. They examined 1132 subjects, 465 of whom had past schistosome infection and found a negative relationship to all components of the metabolic syndrome tested, although the statistical relevance of the relationship with blood glucose levels was reduced under multivariate analysis.

In 2015 Wiria et.al. tested 646 adults in Indonesia for a relationship between Soil Transmitted Helminth (STH) infection and insulin sensitivity[49]. Subjects were tested for *A. lumbricoides*, *T. trichuria, A. duodenale, N. americanus* and *S. strongyloides* and their insulin levels and homeostatic model assessment for insulin resistance (HOMA-IR) were calculated. A significant reduction in HOMA-IR, mostly attributable to lower insulin levels, was found in infected subjects. The relationship was found to correlate with the number of infections present, and it remained significant when adjusted for BMI.

The same group of researchers in Indonesia announced the first prospective trial to examine the effect of treatment for helminth infection on insulin resistance in 2016. The "SUGARSPIN" trial was a household based, double blind, cluster-randomized trial of treatment with albendazole for STH infection and its effect on insulin resistance in a population on Flores island Indonesia, where STH infection was common. Results of this study were published in 2017[50] and failed to show an effect of albendazole treatment on insulin resistance (IR) at a community level. However, when only those who were found to be infected with helminths were included, a significant effect on HOMA-IR was recorded (increase in IR 0.031, confidence interval 0.004-0.059, p=0.04). The authors note that the treatment effect on IR was only moderate, and that it was attenuated by adjustment for changes in BMI. They speculate that this, and the absence of effect at a community level, could be due to the relatively short period of follow up, and the possibility that past infections with helminths were exerting an ongoing effect on IR, as has been suggested by past observational studies. It should also be noted that *S. stercoralis* infection in this community was measured as less than 1% by faecal PCR testing and appeared to disappear entirely after albendazole treatment. Faecal PCR testing for strongyloides, while more sensitive than direct parasitological tests, may still have low and variable sensitivity on single specimens[27]. Studies elsewhere suggest that short courses of albendazole are likely to be ineffective as a treatment for strongyloides[2, 28]. If the study failed to accurately diagnose *S. stercoralis* infection, or indeed infection with any other helminths, then this would have the effect of diluting any observed effect and may partially account for the absence of any observed effect at a community level. Nevertheless, this study represents the first prospective study of its kind, and seems to confirm the hypothesis that treatment of STH may have an effect on IR.

1.2.3 *Helminth infections and other metabolic outcomes*

In addition to studies that look directly at T2DM and the metabolic syndrome, there have been several which look at further outcomes, in particular the atherogenic index, the presence of atherosclerosis, and the rate of cardiovascular disease. In general, these studies have found a less constant relationship with helminth infection.

Aravindhan and the CURES study examined the relationship between LF infection and coronary artery disease in 453 subjects and found no relationship[51]. They further found no relationship with intimal medial thickness and serum CRP levels in the same group, and no evidence of protective immunomodulation.

An Indonesian study looked at STH infection and a range of atherogenic indicators along with carotid intimal medial thickness[52]. They found a negative effect of helminth infection on CVD risk factors such as total cholesterol, BMI and waist/hip ratio, but no relationship to carotid intimal medial thickness.

In contrast, a Russian study examined the relationship between chronic infection with the liver helminth *Opisthorchis felineus* and aortic atherosclerosis in autopsy specimens[53]. Three hundred and nineteen cadavers were examined and a linear negative association described between worm burden and both atherosclerosis and total cholesterol levels. Interestingly, data with regard to T2DM were recorded as part of the study, but did not show any relationship to worm infection or burden. This may have been partly due to the preponderance of young, otherwise fit, males in this autopsy study.

A Chinese study of 1597 men aged over 45 years found a significant reduction in the atherogenic index of plasma (AIP), triglycerides, waist circumference and body mass index for those with a history of past schistosomiasis compared to controls[54]. The relationship to the AIP persisted when adjusted for BMI and waist circumference, suggesting that past schistosomiasis was an independent risk factor.

In 2017 the SUGARSPIN group reported on the outcome of treatment for helminth infections with albendazole, and the adipokines leptin, adiponectin and resistin[55]. These metabolically active hormones are thought to be involved in the regulation of metabolism and insulin sensitivity, and resistin levels have been implicated in the outcome of treatment for helminth infections[56]. Adiponectin is known to be associated with improved insulin sensitivity, while leptin and resistin are thought to be associated with increased insulin resistance. The study

showed a significant increase in the ratio of leptin to adiponectin (treatment effect factor (95% confidence interval), P-value for interaction: 1.20 (1.06-1.35), P=0.010), due largely to an decrease in adiponectin levels, in helminth infected individuals after treatment with albendazole. The effect was thought to account, at least in part, for the effect on insulin sensitivity seen in their earlier study. No effect was demonstrated in resistin levels.

1.2.4 *Strongyloides and T2DM*

Prior to the studies constituting the subject of this thesis there was little in the literature in regard to T2DM and *Strongyloides stercoralis*.

A study in India in 2011 examined the risk factors for *Strongyloides stercoralis* infection in a hospital population[57]. It was a retrospective study employing case record reviews, and looked at *S. stercoralis* infection as an outcome. Its principal concern was with HIV infection, but it does record T2DM as a potential risk factor. Although there were only 22 T2DM patients out of 358 subjects, a statistically significant negative association (OR 0.19, CI 0.04-0.83) with strongyloides infection goes uncommented upon in the study.

The only paper to report upon a positive relationship between helminth infection and T2DM was a small observational study conducted in a Brazilian endocrinology clinic in 2006[58]. 78 diabetic patients were compared to 42 controls with other endocrinological conditions, and a positive association with *S. stercoralis* infection was described (OR 3.9, CI 1.6-15.9). In addition, diabetic patients with a lower HbA1c were found to be less likely to have *S. stercoralis* infection. This study is notable as it stands alone as the only study to find a positive association between helminth infection and T2DM, and the reason for this, apparently contrary, finding is not immediately clear. The study relies on the presence of current infection in a region described as "hyper-endemic" for *S. stercoralis* infection, and no history of past anthelminthic treatment is given. Therefore, it is not known whether the subjects have recent infection, longstanding infection, or indeed both. A protective effect for *S. stercoralis* would presumably not be expected to manifest itself if the majority of infections were recently contracted, or in older patients who already had T2DM.

1.3 Thesis Outline

This thesis seeks to examine the relationship between *Strongyloides stercoralis* infection and T2DM in an Australian Aboriginal community. It postulates that a negative relationship will be found between the two conditions, and that evidence will be found in support of an immunometabolic mechanism to explain this finding. In addition, the thesis will necessarily examine issues relevant to the prevalence, diagnosis and treatment of *S. stercoralis* infection, and will test the effectiveness of a "case finding and treatment" strategy in the control of this infection in an Australian Aboriginal community.

Chapter one has dealt with the necessary background information and a review of the current literature on this subject. Chapter two reports on a cross sectional observational study of adults attending the health centres in the study community, testing them for both strongyloides infection and T2DM, and will establish the nature of the relationship between these two conditions. This will then establish a cohort of infected and un-infected subjects who can be followed over a three-year period in order to determine the outcomes of treatment, and enabling characterization of both the initial relationship between the two diseases, and the effects that treatment for strongyloides may have on subsequent metabolic parameters. Chapter three will examine the outcome at 6 months follow up in terms of the success of treatment for *S. stercoralis* and the presence of any factors, including T2DM, which might impact on this success. Chapter four will then examine the relationship of peripheral eosinophilia to strongyloides infection, both in regard to the predictive value of eosinophilia in the diagnosis strongyloides infection, and its relationship to T2DM.

In a study such as this it is also important to acknowledge that *Strongyloides stercoralis* is a significant and potentially fatal infection for people living in these communities. There are continuing efforts, both in Australia and internationally, to improve the diagnosis, treatment and follow up of this condition, and to decide upon which strategies are likely to be most effective in the control and elimination of the infection from affected communities. In addition to issues of metabolic outcome, any study dealing with *S. stercoralis* is therefore obliged to deal where possible with these issues of diagnosis, treatment, follow up and community wide control. Chapter five will deal explicitly with this topic, and examine the outcomes in terms of treatment success at the three-year follow up of the cohort.

Finally, Chapter six will look at the metabolic outcomes after three years for patients treated for strongyloides, both for patients with pre-existing diabetes and those without, and with special consideration for those in whom treatment was not successful. The thesis will conclude with a discussion of the context and possible consequences of the findings in terms of both the strategies used to control *S. stercoralis* infection, and the effect that helminth infection has on glucose metabolism. As well as examining the central issue of the relationship between *Strongyloides stercoralis* infection and T2DM, the design of this study will allow questions regarding the prevalence of infection, the utility of ELISA testing in diagnosis and follow up, factors effecting treatment failure or success, and the effectiveness of a "case finding and treatment" strategy in the longer-term control of infection to be answered in the context of this community. This may then have broader implications for treatment and control of the infection both within Australia and worldwide.

Each of Chapters two to six will constitute a separate peer reviewed published paper. References for each chapter have been rationalized and combined at the end of the thesis to minimize repetition.

Chapter 2 *Strongyloides stercoralis* Infection and T2DM: Evidence from an Australian Aboriginal Community

2.1 Introduction

The first stage in this thesis required the establishment of a cohort of patients in a community where both T2DM and infection with *Strongyloides stercoralis* were prevalent, in order to demonstrate any association between these two conditions. The Indigenous communities of northern Australia are an ideal setting for a study of this kind, with an extremely high prevalence of both conditions occurring in a setting where adequate resources exist for the appropriate diagnosis, treatment and follow up of patients.

This cross sectional, observational study was, in itself, a project without precedent at this time. As noted, prior to the commencement of this study, very little had been published with regard to the relationship between helminth infection and type 2 diabetes mellitus, and the few studies that had appeared had all treated T2DM as the *exposure* and helminth infection as the *outcome*.[45, 46, 57, 58] Furthermore, the one published study that had looked at the relationship between *Strongyloides stercoralis* infection and T2DM, conducted in a Brazilian endocrinology clinic and involving only small numbers of patients, found a positive association between the two conditions[58]. To this time, it remains the only study that has found a positive association between helminth infections and metabolic disease, suggesting that the population studied may have been in some way biased. The diabetic patients recruited to this study were a hospital based sample and therefore are unlikely to be representative of the wider diabetic population. It seems probable that T2DM patients recruited from a hospital endocrinology clinic would be biased toward those with relatively severe and poorly controlled disease, and as we shall see, this may in part explain the study findings.

In contrast to this clinical study, laboratory investigations in 2010 and 2011 had given support to the theory that chronic helminth infection may result in improved glucose metabolism. Models demonstrating that eosinophilic infiltration of adipose tissue, as a result of experimental helminth infection, sustained the presence of alternatively activated macrophages which in turn increased insulin sensitivity in peripheral tissues through the action of anti-inflammatory cytokines suggested that chronic helminth infection may produce similar changes in a human population[8, 9].

The survey was conducted in 2013 and 2014, and the results were published in early 2015.

Hays R, Esterman A, Giacomin P, Loukas A, McDermott R, 2015. Does *Strongyloides stercoralis* infection protect against type 2 diabetes in humans? Evidence from Australian Aboriginal adults. Diabetes Res Clin Pract 107: 355-61.

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2.2 Does *Strongyloides stercoralis* Infection Protect Against Type 2 Diabetes in Humans? Evidence from Australian Aboriginal adults

2.2.1 *Abstract*

Objective: To explore the relationship between infection with *Strongyloides stercoralis* and the likelihood of having type 2 diabetes mellitus (T2DM).

Methods: Cross-sectional survey of 259 Aboriginal adults living in a remote community in northern Australia during 2013. Prior infection with *S. stercoralis* was determined by ELISA testing on serum. Main outcomes were eosinophil count, T2DM diagnosis, HbA1c, BMI, fasting lipids, Hb, blood pressure.

Findings: Ninety-two participants (36%) had prior infection with *S. stercoralis* and 131 (51%) had T2DM. Those with previous *S. stercoralis* infection (ELISA titre ≥0.3) were 61% less likely to have a diagnosis of T2DM than those uninfected, adjusted for age, triglycerides, blood pressure and BMI using propensity score (adjusted OR=0.39, 0.23-0.67, p=0.001).

Interpretation: In this remote community where prevalence of both *S. stercoralis* and T2DM is very high, infection with *S. stercoralis* appears to significantly reduce the risk of T2DM in adults. A plausible immunological mechanism has been identified in animal models. If confirmed, this result may have practical implications for the prevention of T2DM and associated metabolic disorders in humans. This finding should be explored further with animal models and with larger longitudinal studies in transitional populations where the risk of both conditions is high.

Funding: No external funding was required for this study.

2.2.2 *Introduction and background*

Strongyloides stercoralis (*S. stercoralis)* is a soil transmitted helminth (STH) infection that affects an estimated 30 to 100 million people worldwide, mostly in the developing world. The worm is transmitted through skin contact with faecal matter or contaminated soil containing the infective larva. Once established *S. stercoralis*, uniquely amongst STH infections, has the capacity for an auto-infective cycle, enabling the development of chronic infection even in the absence of further exposure. The fact that it may be asymptomatic for many years, or even decades, means that even amongst the neglected STH infections it has remained neglected. Historically, difficulty in establishing a gold standard for the diagnosis

of infection, and the relative absence of effective treatment has hindered both research into and treatment of this condition[14, 24].

Many of the developing countries where *S. stercoralis* is prevalent are now facing an approaching epidemic of obesity and type 2 diabetes mellitus (T2DM), however it is currently unclear whether these conditions are interrelated. Previous research has explored the potential relationship between helminth infection and chronic inflammatory diseases such as asthma, inflammatory bowel disease, and in particular, Type 1 diabetes mellitus (T1DM)[59, 60]. While a negative association between helminth infection and T1DM has been described, research into the relationship between T2DM and helminth infection has been limited by the fact that these two conditions, in general, only co-exist in high prevalence in "transitional" societies, where access to the necessary tools for the diagnosis of infection is often limited. There is however, a growing body of evidence from studies using experimental animal models to suggest that helminth infection is able to affect the development of metabolic illnesses such as insulin resistance and T2DM through a process of immunomodulation[9, 61].

Very little research attention has been given to the possible interaction between *S. stercoralis* and T2DM in clinical studies. A small observational study conducted in Brazil suggested a positive association between the two conditions[58]. A larger study of risk factors for the presence of *S. stercoralis* in India, however, suggested the opposite[57]. A further study in India examined the relationship between T2DM and another chronic nematode infection, lymphatic filariasis (LF), and found a negative association. In addition, they found evidence for an altered immune response in diabetic patients with LF[46].

The Aboriginal communities of Northern Australia are unique in that they are home to some of the highest recorded prevalence of *S. stercoralis* infection in the world[34] in addition to being hyper-endemic for T2DM[62]. The communities of the east Kimberley region are no exception to this. A survey of one community in 1990 registered a prevalence of 37% for *S. stercoralis* infection[63]. A recent audit of the clinic records suggested that between 20 and 30% of adults across the region have T2DM, and that approximately 50% of all adult patients presenting to the community clinics have a diagnosis of T2DM (Internal audit 2012). This study setting therefore provides a possibly unique opportunity in which to examine the relationship between these two chronic illnesses. We explore here the relationship between *S. stercoralis* infection and T2DM in a transitional population where the incidence of both conditions is high.

2.2.3 *Methods*

2.2.3.1 Study setting

The study communities comprise three related settlements within a 100km radius on the edge of the Tanami desert, about 800 km east of the town of Broome. The population is nearly exclusively indigenous and numbers approximately 1500 individuals spread between the three communities. Medical services are provided through the Kimberley Aboriginal Medical Services Council, which is centred in Broome. This is also the location of the nearest hospital and laboratory services

2.2.3.2 Patients

Prior to this study very little testing or treatment of *S. stercoralis* had taken place in the study communities. There was a well-established practice of giving patients short courses (one to three days) of albendazole 400 mg for presumed helminth infection, and the occasional dose of ivermectin had been given on an empirical basis. In April 2012 the decision was made to begin testing and treating patients for *S. stercoralis* according the best practice guidelines of the Australian Strongyloides working group[32]. Accordingly, patients attending the clinic were offered testing and treatment for the infection on an opportunistic basis. As the presumed prevalence of *S. stercoralis* in the region was about 37%[63], all Indigenous patients resident in the study communities were considered to be at risk of infection.

Data was extracted including the age, sex, date of testing, *S. stercoralis* ELISA titre, haemoglobin, total eosinophil count, percentage eosinophilia, height, weight, calculated BMI, diabetic status and HbA1C Triglyceride level, HDL and total cholesterol. Eosinophilia was defined as a total eosinophil count of 0.50 $x10^9$/L or greater. In addition, data was recorded regarding the past treatment, if any, with anthelmintic drugs. Patients were excluded from the study if they had received past treatment with ivermectin in the absence of serological testing.

2.2.3.3 Serological testing

S. stercoralis ELISA testing was ordered, often in conjunction with other routine laboratory investigations. Serological testing for *S. stercoralis* using ELISA has been shown to be adequate for both diagnostic and sero-survey purposes[25]. Testing was performed by Pathwest Laboratory in Perth Western Australia, using the commercial strongyloides IgG ELISA (DRG laboratory). The reference values, in units of absorbance, given for this test were as follows: Less than 0.2 –Negative; 0.2 to 0.4 –Equivocal; Greater than 0.4 –Positive. However, as was noted by the laboratory, these ranges were developed in a metropolitan

population where the prevalence of *S. stercoralis* was 1/10000 individuals. In order to reduce the possibility of false negative results it was decided to employ a modified range. All values greater than or equal to 0.30 were considered positive and treated. All values less than 0.30 were considered equivocal and were re-tested after a period of 6 months. As the level of 0.30 was chosen purely on clinical grounds as a means of determining when to treat, analysis of the relationship between strongyloides serology and T2DM was repeated at both lower (0.20) and higher (0.40) cut-off points. The case definition for *S. stercoralis* infection therefore was an Aboriginal adult older than 20 years, resident in the study communities, with a *S. stercoralis* ELISA titre of 0.30 or greater.

2.2.3.4 Determination of T2DM status

Diabetes was defined in this group as an HbA1C reading of 6.5% or greater, or a random blood glucose of more than 11.1 mmol/l, or a fasting blood glucose of more than 7.0 mmol/l, either at the time of testing, or in the past in patients already receiving treatment for diabetes.

2.2.3.5 Anthelminthic treatments

Patients identified as positive for *S. stercoralis* were treated with two doses of ivermectin 0.2 mg/kg given two weeks apart, and were recalled for re-testing after a period of 6 months post treatment. All patients identified as equivocal were recalled for repeat testing after a period of 6 months.

2.2.3.6 Regulatory commitments

The protocol for this study was approved by the Kimberley Aboriginal Health Planning Forum. Ethical approval for the study was obtained through the Western Australian Aboriginal Health Ethics Committee (HREC Reference 515).

2.2.3.7 Funding

No external funding was required for this project

2.2.4 *Statistical analysis*

Descriptive statistics included mean, medians (where data was not normally distributed) and percentages, and their respective 95% Confidence Intervals (CI). Comparisons of variables between those with and without diabetes used independent samples t-tests and Mann-Whitney U-tests for continuous variables, and chi-square tests for categorical variables. 95% CI for medians was obtained by bootstrapping. The association between being ELISA positive for *S. stercoralis* and diabetes was assessed using logistic regression. A propensity

score was first created using age, BMI, triglycerides and systolic blood pressure as predictors of positivity. Multiple imputation using five sets of data and assuming a multivariate normal model was then used to impute missing propensity scores. Age, sex, systolic and diastolic blood pressure were used for the imputation process. Finally, the odds ratio adjusted for the propensity score was obtained based on the pooled results. All analyses were undertaken using STATA v13.

2.2.5 *Results*

Approximately half (51%) of the subjects included in the study had T2DM and 36% had evidence of *S. stercoralis* infection (ELISA titre >=0.3) (Table 2.1). There were significant differences in those participants who had T2DM and those who did not. As expected, those with T2DM were older, heavier, had higher blood pressure and higher triglycerides (Table 2.2). Interestingly, those with T2DM were 61% less likely to have a positive ELISA test for *S. stercoralis* (crude odds ratio 0.37, 95% CI 0.22-0.62) (Table 2.3). This relationship remained after adjustment for age, SBP, triglycerides and BMI (adjusted odds ratio 0.39, 0.23-0.67; p=0.001). The relationship also persisted when the cut-off point for diagnosis of strongyloides was set at either 0.20 or 0.40 units.

Amongst the 92 cases where *S. stercoralis* infection was identified, there was a significantly higher incidence of eosinophilia in individuals with diabetes compared with the non-diabetics (Table 2.4).

Follow up of the "equivocal" group over an average of 228 days revealed only 3 new cases in this period, and demonstrated an excess of falling or steady *S. stercoralis* titres compared to rising titres, [Falling (or steady) titre 66.4%, Rising titre 33.6%] and a significant overall fall in the average titre [Median Antibody titre (95% CI) Pre 0.08 (0.08 – 0.10) Post 0.06 (0.05 – 0.07); p = <0.001].

Table 2.1 Clinical characteristics of the study participants (N=259).

Variable	N	Mean, Median or %	95% CI
Age (years)	259	43.4	41.7 - 45.1
Male	106	40.9%	35.1 - 47.1
Weight (Kg)	253	81.6	78.9 - 84.4
BMI (kg/m2)	245	29.7	28.7 - 30.6
Hb (g/l)	249	133.8	131.7-135.8
HbA1c %	220	6.8[a]	6.5 - 7.1
SBP (mmHg)	259	126.8	124.5 - 129.1
DBP (mmHg)	259	79.7	78.3 - 81.1
Cholesterol (mmol/l)	228	4.6	4.4 - 4.7
HDL (mmol/l)	227	0.90	0.87- 0.93
Triglycerides (mmol/l)	227	2.1[a]	1.9 - 2.3
Diabetes	131	50.6%	44.5 - 56.7
Eosinophil count	237	0.43[a]	0.38 - 0.48
Eosinophil %	236	5.55[a]	4.71 - 6.39
E (ELISA) titre	259	0.15[a]	0.12-0.18
% E-titre ≥ 0.3	92	35.5	29.7 - 41.7

[a] Median

Table 2.2 Characteristics of participants by diabetic status.

	Not diabetic (N=128)	**Diabetic (N=131)**	**Sig.[a]**
Mean (95%CI) Age (years)	40.4 (37.9 - 43.0)	46.3 (44.0 - 48.5)	<0.001
Gender %			0.268
Male	37.5 (29.5 - 46.3)	44.3 (35.9 - 52.9)	
Female	62.5 (53.7 - 70.5)	55.7 (47.1 - 64.1)	
Mean (95%CI) Weight (Kg)	76.2 (72.5 - 79.9)	86.8 (82.9 - 90.7)	<0.001
Mean (95%CI) BMI (kg/m2)	28.0 (26.7 - 29.4)	31.2 (29.9 - 32.6)	0.001
Mean (95%CI) Hb (g/l)	133.7 (130.6-136.6)	133.9 (131.0-136.9)	0.878
Median (95%CI) HbA1c %	5.8 (5.7 - 5.9)	8.1 (7.7 – 8.5)	<0.001
Mean (95%CI) SBP (mmHg)	122 (120 -125)	131 (128 - 135)	<0.001
Mean (95%CI) DBP (mmHg)	78 (76 - 80)	82 (80 - 84)	0.095
Median (95% CI) Tot. Chol. (mmol/l)	4.4 (4.1 - 4.7)	4.3 (4.0-4.5)	0.197
HDL (mmol/l)	0.92 (0.87 - 0.96)	0.88 (0.84 - 0.92)	0.260
Median (95% CI) Trig. (mmol/l)	1.7 (1.4 - 2.0)	2.6 (2.3 - 2.9)	<0.001
ELISA			<0.001
<0.3	53.1 (44.4 - 61.7)	75.6 (67.4 - 82.2)	
≥0.3	46.9 (38.3 - 55.6)	24.4 (17.8 - 32.6)	
Eosinophils	0.41 (0.35 – 0.47)	0.44 (0.38 – 0.50)	0.236

[a] Independent samples t-test or Mann-Whitney U-test for continuous variables, and chi-square test for gender and ELISA category

Table 2.3 Association between positivity for *S. stercoralis* and diabetic status.

Serology cut-off	Variable	Odds Ratio (OR)	95% CI (OR)	Sig.[a]
≥0.3	Positivity	0.37	0.22 – 0.62	<0.001
	Positivity[b]	0.39	0.23 – 0.67	0.001
≥0.2	Positivity	0.49	0.30 – 0.81	0.005
	Positivity[b]	0.49	0.30 – 0.83	0.007
≥0.4	Positivity	0.42	0.24 – 0.73	0.002
	Positivity[b]	0.43	0.25 – 0.75	0.003

[a] From logistic regression
[b] Adjusted for Age, triglycerides, systolic blood pressure and BMI using a propensity score

Table 2.4 Characteristics of ELISA Positive patients by diabetic status.

Variable	Non-Diabetic (N=60)	Diabetic (N=32)	Sig.
Mean (95% CI) Age	41.2 (36.9 – 45.6)	46.8 (42.7 – 50.9)	0.098[a]
Gender - male	35%	50%	0.162[c]
BMI	27.8 (28.3 – 35.4)	31.8 (28.3 – 35.4)	0.031[a]
Eosinophilia	55.5%	71.9%	0.110[c]
Median (95% CI) eosinophil count	0.54 (0.42 – 0.65)	0.78 (0.47 – 1.09)	0.020[b]
Median (95% CI) Antibody titre	0.57 (0.37 – 0.77)	0.86 (0.66 – 1.07)	0.244[b]
Median (95% CI) Triglycerides	1.70 (1.29 – 2.11)	2.05 (1.44 – 2.66)	0.105[b]

[a] Independent samples t-test
[b] Mann-Whitney U-test
[c] Chi-squared test

2.2.6 *Discussion*

Our study found a strong negative association between *S. stercoralis* infection and T2DM in this community. This negative association persists when corrected for age, gender and body mass index. The obvious limitation of this cross sectional observational study is that causality cannot be implied. However, knowledge of the natural history and pathophysiology of *S. stercoralis* infection and T2DM can assist in developing some explanation for these observations.

As noted above, *S. stercoralis* is able to produce a chronic, self-sustaining infection. The proportion of acute infections that progress to this chronic stage is, however, unknown. Studies have demonstrated that, as with other STH infections, the prevalence increases until late adolescence and then remains relatively constant throughout adult life[13].

T2DM on the other hand, is a metabolic illness that begins to manifest clinically in late adolescence, and then increases in prevalence progressively into mid-life. It is difficult to conceive of a mechanism by which the presence of T2DM might protect against *S. stercoralis* infection, or prevent the development of a chronic infection.

A more plausible explanation for the data is that the presence of chronic *S. stercoralis* infection can, over time, protect against the development of T2DM.

The question as to whether the infections are acute or chronic is clearly important. There is no established protocol by which the chronicity of any particular strongyloides infection can be determined. Clinical assessment in communities where the infection is common is of little value as the onset of infection is often not apparent, or produces only non-specific symptoms, and patients with long standing infections are often symptom free[64].

Some conclusions may be drawn from the rate of acquisition of infection in this community. The 167 patients who were initially negative for *S. stercoralis* infection were followed up for an average of 287 days, and in this time only 3 patients acquired the infection according to the criteria used in this study. This would suggest that the acquisition rate for *S. stercoralis* amongst previously uninfected adults is relatively low and therefore imply that the majority of infections seen here are longstanding in duration.

Follow up of the negative patients demonstrates a preponderance of falling or steady titres compared to rising ones, and an overall significant reduction in the average titre over the follow up period. This could suggest that negative patients with measurable titres in fact represent patients who have been exposed to the parasite in the past, but in whom a patent chronic infection has not been established.

Given that the data in this study suggests that chronic infection with *S. stercoralis* can protect against the development of T2DM, what mechanisms can be suggested to explain this observation?

A simple, nutrition-based explanation, where the worm competes with the host would seem unlikely. It is not born out by the data, as the association is unrelated to BMI. It would also seem unlikely on pathophysiological grounds. Chronic infections with *S. stercoralis*, in the absence of hyperinfection, are known to comprise only very low numbers of adult worms[13] each only about 2 mm long, suggesting that the nutritional burden of chronic infection is likely to be low. Gastrointestinal symptoms, in particular chronic diarrhea and mal-absorption have been described mainly in acute infections and in children, and are not commonly reported in chronic infections, particular in communities where the infection is common[64].

It is plausible that the presence of strongyloides infection may have an effect on the microbiota of the gut and influence the prevalence of T2DM in this way. It is known that the gut microbiota is altered in diabetic patients compared to non-diabetic[41] and animal studies have demonstrated the effect that helminths may have on gut bacteria[65]. Recently experimental hookworm infection in humans has been shown to cause an altered microbiota in some instances[66].

Numerous studies have now demonstrated that the development of T2DM in adult life is more common among low birth weight (LBW) babies[67]. Furthermore, this association is strongest amongst LBW babies who subsequently gain weight rapidly in the first decade of life[68]. Interestingly, longitudinal studies underway amongst Indigenous populations in northern Australia have yet to demonstrate this association, and it has been postulated that this may be because the population studied is failing to gain weight in early life[69]. Australian Aboriginal children commonly suffer numerous parasitic and bacterial infections early in life, which may contribute to this lack of rapid weight gain. It is plausible that *S. stercoralis* infection, acquired early in life and leading to a subsequent chronic infection, might contribute to lack of weight gain and failure to thrive in the first decade of life, and subsequently reduce the incidence of T2DM later in life. Clearly, studies that examine the prevalence of *S. stercoralis* infection in children in these communities and its association with weight gain, BMI and nutritional status, would be of great benefit.

An equally compelling explanation for the observed association is that chronic *S. stercoralis* infection is able to reduce the incidence of T2DM through a process of immunomodulation. Chronic helminth infections elicit a modified T helper type 2 (Th2) immune response in the host mediated through a reduction in pro-inflammatory cytokines such as IFN-γ and TNF-α and an increase in anti-inflammatory cytokines such as IL-10 and TGF-β, thereby promoting

the survival of the parasite and reducing the risk of inflammatory injury in the host[70]. There is also evidence that immunomodulation may influence the development of metabolic illnesses such as insulin resistance and T2DM. Clinical studies have suggested that the occurrence of T2DM is related to the overall level of inflammation present over time[71]. Conversely there are studies that demonstrate a negative association between IL-10 and T2DM[72]. If chronic infection with *S. stercoralis* were responsible for producing a low inflammation environment in the host then this could explain the subsequent lower incidence of T2DM.

The association between chronic inflammation in adipose tissue and the development of insulin resistance and T2DM has been long established[35]. This inflammation is mediated through the Th1 immune response and "classically activated" macrophages, and involves increased levels of pro-inflammatory cytokines such as TNF and IL-1β. Conversely, in lean individuals adipose tissue is infiltrated by alternatively activated macrophages (AAM) that reduce inflammation and promote insulin sensitivity through an IL -10 and regulatory T cell-mediated process[9]. Experimental studies have shown that eosinophils infiltrating adipose tissue are central to recruiting and maintaining AAM through IL-4 and IL-13, and that Th2 responses and eosinophilia in mice with experimental helminth infections are capable of increasing insulin sensitivity[8].

In our study, we observed a significantly higher eosinophil count amongst the patients with T2DM compared to those without. As this is a cross sectional observational study, causality cannot be implied. The observation could be explained if the immune response amongst diabetics were for some reason more vigorous than that that amongst non-diabetics. However, since the rate of eosinophilia and the overall eosinophil count in *S. stercoralis* infection tend to be higher in early infections and diminish with the duration of infection[73], a more likely explanation would be that the T2DM group contains, on average, more acute or recent infections and consequently less chronic infections than the group without T2DM.

It is possible therefore to propose a model where acute infection with *Strongyloides stercoralis* and the subsequent Th2 immune response leads either to worm killing and resolution of the infection in some cases, or the establishment of a chronic well adapted infection, with a modified Th2 immune response, ongoing infiltration of adipose tissue with eosinophils and AAM's, and a subsequent reduction in insulin resistance in others (Figure 2.1).

In the first instance the resolution of infection leads to a return to baseline levels of risk for T2DM, while the presence of chronic *S. stercoralis* infection in the other group results in a lower prevalence of T2DM.

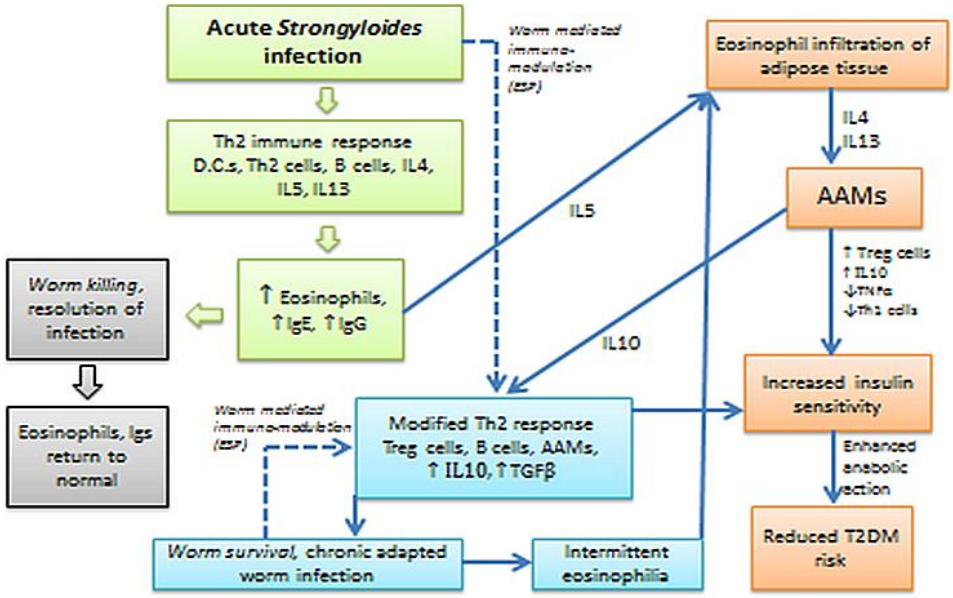

Figure 2.1 Potential immuno-metabolic pathways for Strongyloides infection and T2DM risk.

2.2.7 *Conclusion*

This study provides, for the first time, evidence that chronic infection with *S. stercoralis* may protect against the development of T2DM in humans. In doing so it provides clinical and epidemiological evidence in support of the animal models that show that helminth infections can result in an improved metabolic profile through a process of immunomodulation.

Increasing attention is being given to the possible role of anti-inflammatory treatments in the management of established T2DM and the importance of "immuno-metabolism" in this process, but attention has thus far concentrated on treatment rather than prevention[74].

Immunomodulation by helminth infections is mediated by excretory/ secretory proteins (ESP) either elaborated by the worm, or present on its surface. Studies have identified many of these proteins in various helminth infections, and in some cases the mechanism by which they produce their effect[75]. If the effect of *S. stercoralis* infection on metabolic processes can be confirmed, and a protein or proteins identified which are responsible for this effect, then it

could provide the prospect of developing a therapeutic agent capable of preventing or delaying the onset of T2DM in susceptible populations.

The authors report no conflicts of interest. We would like to formally acknowledge the role of the peoples of the Kutjungka region in this study.

Chapter 3 *Strongyloides stercoralis* Infection and T2DM: Implications for Treatment Outcomes

3.1 Introduction

Having established a relationship between *Strongyloides stercoralis* infection and T2DM in a cross sectional observational study, the opportunity then exists to follow the cohort of infected and uninfected patients in a longitudinal study. This would enable examination of the effect of prior T2DM on treatment outcomes, the long-term effect that treatment of adults would have on the prevalence of infection in the community, and the effect that treatment for strongyloides infection might have on the incidence and control of T2DM in this population.

The initial follow-up of our cohort was conducted after 6 months, when changes in ELISA serology levels could be expected to reflect the success or otherwise of treatment. Metabolic outcomes were not measured after this relatively short time period as it was thought unlikely that any appreciable effect on diabetic outcomes would be apparent. The outcome of treatment however, and any relationship it might have to prior T2DM status should be apparent after 6 months, which is within the usual follow up period recommended to demonstrate a fall in strongyloides ELISA titre, and therefore treatment outcome[26]. If a relationship could be established between T2DM and treatment outcome then this would give support to the theory that an interaction exists between T2DM and the immunological reaction to *S. stercoralis* infection. Clinical experience holds that bacterial and viral infections are often more difficult to control in patients with T2DM, but no such relationship has been demonstrated in helminth infections.

This study was also of interest to clinicians concerned with the control of *S. stercoralis*, as follow up studies of treated strongyloides patients were uncommon both in Australia and internationally, and had been characterized by relatively large numbers lost to follow up after even relatively short time periods[34, 76]. It has been thought that ivermectin is an effective treatment for *S. stercoralis* in this population, but in fact good evidence for this was lacking, and there had been no published studies which examined the effect of factors such as T2DM in the outcome of treatment.

Follow up of our cohort at 6 months post treatment was completed in 2014 and the results were published in early 2015.

Hays R, Esterman A, McDermott R, 2015. Type 2 Diabetes Mellitus is associated with *Strongyloides stercoralis* treatment failure in Australian Aboriginals. PLoS Negl Trop Dis 9: e0003976.

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3.2 Diabetes Mellitus is Associated with *Strongyloides stercoralis* Treatment Failure in Australian Aboriginals

3.2.1 *Abstract*

Objective: To explore the efficacy of ivermectin in the treatment of serologically diagnosed cases of *Strongyloides stercoralis* (*S. stercoralis*) infection in an Aboriginal community and to describe factors that may influence the outcome of treatment.

Methods: Longitudinal study of a group of 92 individuals with serologically diagnosed *S. stercoralis* treated with ivermectin and followed up over a period of approximately 6

months. Main outcomes were serological titres pre and post treatment, diabetic status, and duration of follow up.

Findings: Treatment success was achieved in 62% to 79% of cases dependent on the methods employed for the diagnosis of infection and assessment of treatment outcome. Type 2 Diabetes Mellitus (T2DM) was found to be significantly associated with treatment failure in this group for two of the three methods employed.

Interpretation: Ivermectin has been confirmed as an effective treatment for *S. stercoralis* infection in this setting. T2DM appears to be an independent risk factor for treatment failure in this population, and plausible mechanisms to explain this observation are presented

Author Summary: In this study, we examine the treatment of the intestinal worm infection *Strongyloides stercoralis*, a soil transmitted helminth that is common in the developing world and in the Aboriginal communities of northern Australia. Oral ivermectin is generally accepted as the treatment of choice for this condition. We screened an Aboriginal community for strongyloides infection over the course of 2 years and measured the outcome of treatment in the 92 cases we found. This study measures the success or otherwise of treatment, and looks at whether there are any factors that could influence the rate of treatment failure. The results suggest that ivermectin is an effective treatment for strongyloides in this setting, and that pre-existing Type 2 Diabetes Mellitus is a risk factor for treatment failure, an observation of great interest in Aboriginal communities where the prevalence of both conditions is very high. We discuss some possible mechanisms to help explain this previously unknown relationship.

3.2.2 *Introduction*

S. stercoralis is a soil transmitted helminth infection and as such is a neglected tropical disease. It is thought to have a worldwide prevalence of at least 100 million people, and is endemic in regions where people are in contact with fecal material or contaminated soil. The majority of infections in the inhabitants of endemic regions are thought to be asymptomatic, but it can produce a variety of gastrointestinal and skin symptoms. Unusually, *S. stercoralis* has the capacity for an auto-infective cycle, and in the presence of host immunosuppression hyper-infection syndrome may develop, resulting in severe morbidity and even death.[14]

In the past, the absence of a gold standard for the diagnosis of this infection, uncertainty as to the most effective treatment, and difficulty in establishing whether a cure has been achieved, has posed problems for research into this condition.[24]

Traditional microbiological methods for detecting strongyloides infection, including concentration techniques for fecal examination such as the Baermann and Harada-Mori techniques, and agar plate culture, require the use of repeated, fresh fecal samples, and have been troubled by low sensitivity. [2] This is particularly so when assessing patients for cure, as larval output can be low and intermittent leading both to under-estimates of the prevalence of the infection, and over- estimates of the effectiveness of treatment.[29.]

In recent years serological methods for the detection of strongyloides infection have been developed and advocated as being suitable for both clinical assessment and epidemiological surveys. Furthermore, there is increasing evidence for the use of serological tests to follow up treatment and assess cure.[25]

Various medications have been employed for the treatment of strongyloides infection including albendazole, thiobendazole and ivermectin. It is now generally accepted that ivermectin is the most effective treatment and should be the treatment of choice where available, although questions still remain regarding the best dosing schedule[29].

Similarly, the best means of follow up after treatment and the appropriate time frame in which to do this has yet to be established. Follow up of ELISA serology has employed either a fall from a positive titre to a negative (treatment success), or a proportional fall in titre such that the ratio of post to pre-treatment titres is less than 0.6(treatment effectiveness)[25] The lengthy time frame required for the follow up of cases has often led to high rates of patients lost to follow up.[76.]

Strongyloides infection is extremely common in Indigenous communities of the tropical regions of northern Australia with a prevalence of 41% in some settings.[34 32] Despite increasing access to serological tests and adequate treatment, screening and treatment programs remain intermittent, and little is known about the efficacy of ivermectin under Australian conditions, with a recent review noting the paucity of adequate follow up studies following treatment[76].

In this study, we report on the treatment and follow up of 92 serologically diagnosed cases of *S. stercoralis* infection in a remote Aboriginal community, and the effect of a variety of epidemiological factors and co-morbidities on the outcome of treatment.

3.2.3 *Materials and methods*

3.2.3.1 Study setting

This study was conducted in three isolated indigenous communities located on the edge of the Tanami desert in the Kimberley region of Western Australia. The communities are inter-related and consist of a mobile population of approximately 1500 people. A medical clinic in each community provides primary care and emergency services, and the nearest hospital and laboratory services are some 800km away in the town of Broome.

3.2.3.2 Patients

Prior to this study the approach to testing and treating for helminth infection had been intermittent and largely empirical. Patients suspected of having a worm infection were generally given a three-day course of albendazole 400mg without any laboratory testing, and very little ivermectin had been used. In April 2012 it was decided to adopt the best practice guidelines of the Australian Strongyloides working group[32] and patients attending the clinic were subsequently offered testing and treatment on an opportunistic basis for *S. stercoralis* infection. As the presumed prevalence of *S. stercoralis* in the region was about 37%[63], all Indigenous patients resident in the study communities were considered to be at risk of infection.

Data were extracted including the age, sex, date of testing, *S. stercoralis* ELISA titre, haemoglobin, total eosinophil count, percentage eosinophilia, height, weight, calculated BMI, diabetic status and HbA1C triglyceride level, HDL and total cholesterol.

Eosinophilia was defined as a total eosinophil count of 0.50 $x10^9$/L or greater. T2DM was defined in this group as an HbA1C reading of 6.5% or greater, or a random blood glucose of more than 11.1 mmol/l, or a fasting blood glucose of more than 7.0 mmol/l, either at the time of testing, or in the past in patients already receiving treatment for diabetes.

In addition, data were recorded regarding the past treatment, if any, with anthelmintic drugs. Patients were excluded from the study if they had received past treatment with ivermectin in the absence of serological testing.

The patient group in this study constituted part of that utilized in an earlier published study[77]

3.2.4 *Serological testing*

S. stercoralis ELISA testing was performed by Pathwest Laboratory in Perth Western Australia, using the commercial Strongyloides IgG ELISA (DRG laboratory). The reference values for this test were established in a low prevalence (<1/10000) population, with > 0.40 units of absorption held to be positive, < 0.20 units negative, and values in between considered "equivocal". As this study was being conducted in a presumed high prevalence setting, and in the interests of avoiding false negative results, a modified range was employed. Values greater than or equal to 0.30 units were considered positive and treated. All values less than 0.30 units and greater than zero were considered equivocal and were re-tested after a period of 6 months.

Clinical treatment success was defined as a fall in titre to < 0.30 units. Only patients who failed to achieve this were, where possible, retreated with ivermectin.

3.2.5 *Anthelminthic treatments*

Patients identified as positive for *S. stercoralis* under these criteria were treated under direct observation with two doses of ivermectin 0.2 mg/kg given two weeks apart. All patients, whether positive or equivocal, were recalled for re-testing after a period of 6 months. Although all patients were recalled after 6 months, difficulties in locating subjects for follow up resulted in a wide range (83 to 498 days, median 214 days) of follow up periods.

3.2.6 *Ethics statement*

The process of obtaining community engagement, consent and ethical approval began in 2012-2013. A process of community and institutional consultation and education was undertaken. Staff at the community clinics, including Aboriginal health workers, nursing staff and administrative staff were educated in informal sessions regarding the nature and importance of Strongyloides infection in Aboriginal communities. In addition, key members of the community including elders, the community council and traditional healers were included in the process. Approval of the project was then sought from the KAHPF (Kimberley Aboriginal Health Planning Forum) which is the peak community consultation body in the Kimberley. Approval was received on April 5 2013 and subsequent application for ethical approval was sought from the Western Australian Aboriginal Health Ethics committee (WAAHEC) and received on November 1 2013 (HREC App 515). Finally,

application was made for ethical approval from the James Cook University (JCU) Health ethics committee and was received on September 21 2015. During the course of the project 6 monthly reports on progress were made to the WAAHEC. In addition, with each published paper approval was sought from WAAHEC prior to publication. Copies of each paper were provided to the KAHPF along with "plain language" summaries intended for publication in the KAHPF community newsletter. Lastly, summaries in the form of "talking points" were provided for clinic workers to provide feedback to patients and community members at each stage."

Once the decision had been taken to test and screen for strongyloides was taken, as no tests, treatments or interventions were undertaken apart from those required for the accepted Australian best practice[32] for the diagnosis and treatment of strongyloides infection, and all data was de-identified before storage, verbal consent was considered adequate. Literacy levels in written English are low in the communities studied. All subjects were 21 years of age or older. Verbal consent was obtained by medical and nursing staff at the time of initial testing and stored electronically.

3.2.7 *Statistical analysis*

As the decision to treat at a level of 0.30 units was based only on clinical grounds, and because the best means for determining treatment failure remains uncertain, the data obtained were analyzed in three different ways.

The first definition of treatment failure was an initial titre of ≥0.30 units followed by a subsequent post-treatment reading of ≥0.30 units. In a second analysis failure to achieve treatment "effectiveness", that is a titre ratio ≥0.60 post-treatment compared to pre-treatment, was the measure of treatment failure. Lastly, the analysis was repeated using the conventional ≥0.40 units as the cut-off point for positivity, and ≥0.40 units post treatment as the measure of treatment failure.

Descriptive statistics are provided with means, median, percentages and their 95% confidence intervals. Bootstrapping was used to obtain the confidence intervals for medians, whereas exact binomial confidence intervals are provided for percentages. Comparisons between those with and without diabetes were tested using independent samples t-tests, Mann-Whitney U-tests and chi-squared tests for Normally distributed continuous variables, skewed continuous variables and proportions respectively. Log binomial generalized linear models were used to assess the association between diabetic status and treatment failure after

adjusting for age, sex, initial titre, follow up period and eosinophilia, and HbA1c (%) as a predictor of treatment failure. All analyses were undertaken using the Stata 13 statistical package.

3.2.8 *Results*

The characteristics of the 259 patients in this study, and the follow up for the 92 positive cases are summarized in tables 1 and 2. Follow up serology was performed in 87 of the 92 patients treated (94.6%). Of the 5 patients not re-tested, one was diabetic and 4 were non-diabetic. One patient had deceased, one was in the prison system and 3 were lost to follow up.

Treatment failure as defined by a post-treatment titre of ≥0.30 was seen in 38% of patients. Using the ratio of post-treatment to pre-treatment titre of ≥0.60, 26% of patients had treatment failure. If only those with an initial titre of ≥0.40 and a subsequent reading of ≥0.40 are defined as a failure, then the treatment failure rate was 21%. The percentage of diabetic and non-diabetic patients achieving a post treatment titre of < 0.30 is shown graphically in Figure 3.1.

T2DM was predictive of treatment failure after adjustment for age, sex, initial titre, follow up period, treatment interval and eosinophilia, for both cut off points of 0.30 units (p =0.025) and 0.40 units (p= 0.006). It was not predictive of failure to achieve treatment "effectiveness" (post to pre-treatment ratio of >0.60).

In addition, follow up of the "equivocal" group (initial titre <0.30, N=167) over an average of 220 days revealed only 3 new cases in this period (1. Female age 53, pre titre 0.19 –post 0.30, 2. Female 43, pre 0.15-post 0.43, 3. Female 30, pre 0.18-post 0.30) suggesting that treatment failures were unlikely to represent incidences of re-infection.

Analysis was performed to determine whether HbA1C, measured as a percentage, was predictive of treatment failure (defined as post treatment titre ≥ 0.30) in the diabetic group and revealed only a statistically insignificant positive association (RR 1.085 95% CI 0.963-1.222 p= 0.179).

Table 3.1 Clinical characteristics of the study participants (N=259).

Variable	N	Mean, Median or %	95% CI
Age (years)	259	43.4	41.7 - 45.1
Male	106	40.9%	35.1 - 47.1
Weight (Kg)	253	81.6	78.9 - 84.4
BMI (kg/m2)	245	29.7	28.7 - 30.6
Hb (g/l)	249	133.8	131.7-135.8
HbA1c %	220	6.8[a]	6.5 - 7.1
SBP (mmHg)	259	126.8	124.5 - 129.1
DBP (mmHg)	259	79.7	78.3 - 81.1
Cholesterol (mmol/l)	228	4.6	4.4 - 4.7
HDL (mmol/l)	227	0.90	0.87- 0.93
Triglycerides (mmol/l)	227	2.1[a]	1.9 - 2.3
Diabetes	131	50.6%	44.5 - 56.7
Eosinophil count	237	0.43[a]	0.38 - 0.48
Eosinophil %	236	5.55[a]	4.71 - 6.39
E (ELISA) titre	259	0.15[a]	0.12-0.18
% E-titre ≥ 0.3	92	35.5	29.7 - 41.7

[a] Median

Table 3.2 Characteristics and outcomes for positive cases by diabetes status with 95% CI.

	No diabetes (N=60)	Type 2 Diabetes (N=32)	Sig.[a]
Age (years) Mean	41.2 (36.9 – 45.6)	46.8 (42.7 – 50.9)	0.098
Male (%)	33.3 (22.0 – 47.0)	50.0 932.0 – 68.0)	0.119
Weight (Kg) Mean	74.2 (68.6 – 79.7)	89.4 (68.6 – 79.7)	0.005
BMI (kg/m^2) Mean	27.8 (25.8 – 29.8)	31.8 (28.3 – 35.4)	0.031
Hb (g/l) Mean	130.9 (126.1 – 135.7)	133.4 (126.7 – 140.1)	0.521
HbA1c % Median	5.9 (5.8 – 6.0)	8.2 (7.2 – 9.2)	<0.001
SBP (mmHg) Mean	124.0 (119.5 – 128.6)	134.5 (126.7 – 142.4)	0.014
DBP (mmHg) Mean	77.3 (74.5 – 80.0)	83.1 (79.3 – 86.8)	0.014
Follow up period (Days) Median	215.5 (198.2 – 232.8)	213.5 (185.4 – 241.6)	0.921
Initial serology (Titre) Median	0.57 (0.37 – 0.77)	0.87 (0.68 – 1.05)	0.244
Days between 1st and 2nd dose	19.5 (15.6 – 23.4)	16.0 (12.1 – 19.9)	0.091
ELISA			
Post-treatment ≥0.30 (%)	31.7 (20.3 – 45.0)	50.0 (31.9 – 68.1)	0.085
Post-treatment ratio ≥0.60	25.0 (14.4 – 38.4)	29.0 (14.2 – 48.0)	0.683
Pre-treatment >0.40 Post ≥0.40	16.7 (8.3 – 28.5)	28.1 (13.7 – 46.7)	0.196
Eosinophilia (%)	54.5 (40.6 – 68.0)	71.9 (53.3 – 86.3)	0.789

[a]Independent samples t-tests for continuous variables, Chi-squared tests for categorical variables, Mann-Whitney U-test for skewed continuous variables

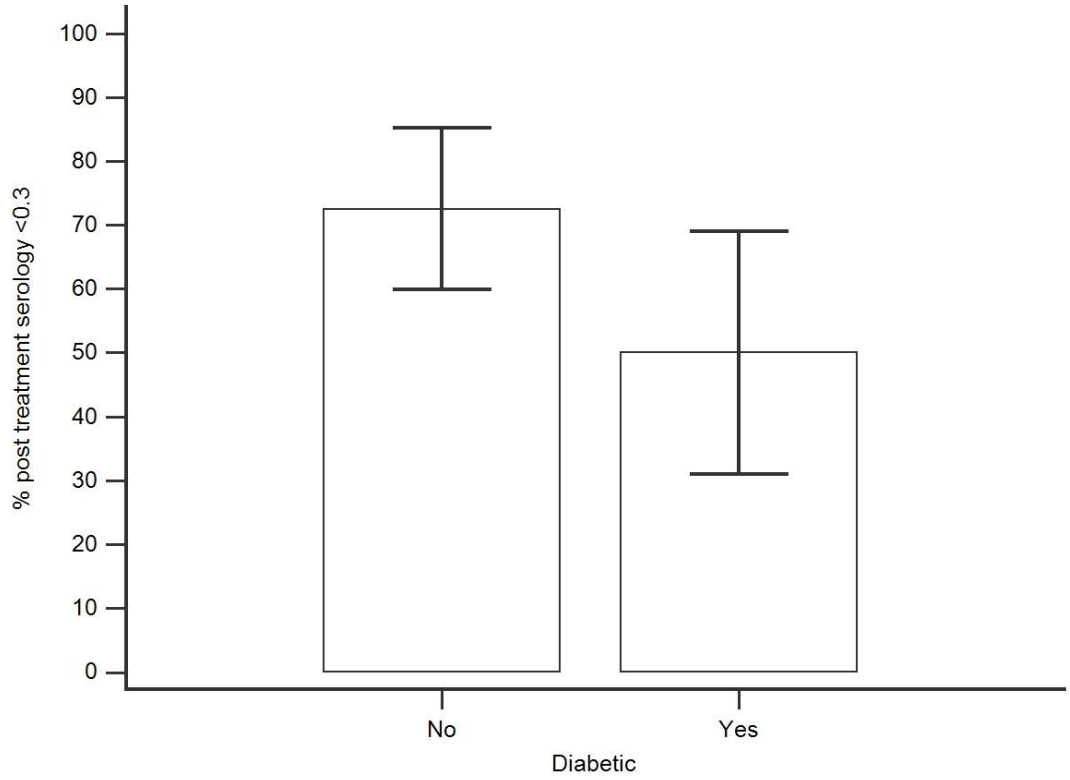

Figure 3.1 Percentage of diabetic and non-diabetic patients achieving a post-treatment titre of <0.30, with 95% confidence intervals.

Table 3.3 Outcomes for positive cases by diabetes status after adjusting for age, sex, initial titre, follow up period, days between first and second dose and eosinophilia.

	Risk ratio (RR)	**95% CI for RR**	**Sig.[a]**
ELISA			
Post-treatment ≥0.30 (%)	1.811	1.076 – 3.048	0.025
Post-treatment ratio ≥0.60	1.482	0.698 – 3.147	0.306
Pre-treatment >0.40 Post ≥0.40	3.848	1.478 – 10.019	0.006

[a]Based on log binomial generalized linear model.

3.2.9 *Discussion*

This study confirms the efficacy of ivermectin treatment for strongyloides infection in the setting of an Australian Aboriginal community, although the overall failure rate for treatment is higher than in some previous studies.[78] The use of serology rather than direct

microbiological examination for follow up, may in part explain this apparent lower efficacy, as it has been noted that the low sensitivity of microbiological tests tend to overestimate the efficacy of treatment.[29] [79]

Reliance upon serology alone for diagnosis, rather than traditional microbiological techniques could be perceived as a potential weakness of this study, as it is likely that microscopy alone is capable of delivering a specificity of 100%. There is increasing evidence however that serology is adequate for diagnosis, sero-surveys and follow up of treatment in this condition[25] , and serology may be superior to microscopy in assessing response to treatment given the very low sensitivity of microbiological techniques.

The high proportion of patients with T2DM in this study (51%) could also be a contributing factor, given that T2DM is here demonstrated to be a risk factor for treatment failure. The low rate of sero-conversion in "equivocal" patients suggests a low incidence of new infections in the community, and therefore that the apparent treatment failures are unlikely to represent subsequent reinfections.

Most notably this study finds that pre-existing T2DM is an independent risk factor for treatment failure when adjusted for age sex, BMI, initial titre, time between treatments, eosinophilia and duration of follow up.

There are several plausible explanations for the effect of T2DM on treatment outcome. Firstly, the possibility exists that drug interactions between T2DM medications and ivermectin, and the pharmacokinetics of ivermectin, may have some impact on treatment failure rates. The most commonly used diabetic medications in this study were gliclazide and metformin. There are no established interactions between gliclazide and ivermectin, and their metabolic pathways suggest that an interaction is unlikely. [80] [81]

However, poor absorption is known to be a problem with oral ivermectin. [7] Hyperinsulinaemia in itself is known to slow gastric emptying [82] and metformin can produce gastric side effects including loose bowel actions. It is therefore plausible that impaired absorption of ivermectin in diabetic patients taking metformin is a factor in the observed association.

It is also feasible that the altered gut biota known to occur in T2DM patients [41]could influence the absorption of ivermectin.

The complex nature of the interaction between helminth infections and T2DM could mean that the nature of the worm infections found in diabetic patients is different. As has been demonstrated elsewhere, helminth infections can in themselves affect glucose metabolism through a process of immunomodulation [8] and data obtained from this community has confirmed a strong negative relationship between *S. stercoralis* infection and T2DM[77]

Consequent to this, it has been suggested that the infections detected in T2DM patients were more likely to represent relatively recent infections (contracted after the onset of T2DM) whereas the infections in non-diabetics were more likely to represent chronic, well-adapted infections with a modified Th2 reaction established in the host. [77] It is known that worm numbers are likely to be higher in early infections and to fall to lower numbers in chronic infections. [13] The higher treatment failure rate in patients with T2DM could therefore be due to a higher average worm burden.

Ivermectin exerts its effect by paralyzing the adult worms, leading to subsequent worm death, and has little effect on the worm larvae. It may be that relatively early infections in patients with T2DM involve higher numbers of auto-infective larvae and that the persistence of these larvae, with the subsequent re-establishment of a patent infection, is responsible for the higher treatment failure.

As is well known, T2DM has been implicated in having a negative effect on both the frequency and outcome of various infections in human beings, through a variety of proposed mechanisms[83, 84] and it may be that the observed association simply represents another example of this process. Though this effect has not to date been described in helminth infections, case histories have been published describing strongyloides hyper-infection syndrome in patients where the only known risk factor was T2DM, suggesting that diabetes can play some role in impairing the immune response to this infection.[22] The fact that treatment failure was found to be only weakly associated with HbA1C suggests that glycemic control in itself may not be the main or only factor at play.

Some laboratory evidence exists to suggest that the immune response to helminth infections in patients with T2DM may play a role. A recent paper[56] has explored the relationship between the adipokine resistin, and multiple helminth infections. The authors found that human resistin was both up-regulated in the period after helminth infection, and that this increased level of resistin was associated with an enhanced Th1 immune response, increased

worm burden and impaired parasite clearance. They conclude that resistin could have significant implications for the outcome of helminth disease in humans and that increased resistin expression could be predictive of impaired immunity to helminths.

This clearly has implications for individuals with T2DM, as resistin levels have been measured in relation to obesity, T2DM and the metabolic syndrome, and are known to be variably increased in these conditions. [85] It may be therefore that the observation that T2DM is a predictor of treatment failure in *S. stercoralis* infection reflects once again the presence of a complex and intimate relationship between this helminth infection, the human immune response, and metabolic illnesses such as T2DM.

3.2.10 *Conclusion*

This study supports the use of ivermectin for the treatment of *Strongyloides stercoralis* infection in an Aboriginal community where the disease is endemic. The association of T2DM with treatment failure in this community suggests that further investigation into the efficacy of ivermectin in this group is warranted. A plausible mechanism to explain this observation involving the adipokine resistin has been described, and further study into the complex relationship between helminth infection, immunity and metabolic disease would be of value.

Regardless of the mechanism responsible, this study suggests that clinicians should be vigilant when treating T2DM patients for strongyloides infection, ensuring an optimal treatment regimen and careful follow up for these individuals.

3.2.11 *Acknowledgments*

The authors would like to acknowledge the role of the peoples of the Kutjungka region in this study.

Chapter 4 Strongyloides, T2DM and Eosinophilia in an Aboriginal Community

4.1 Introduction

As well as characterizing the relationship between T2DM and *S. stercoralis* infection, the data obtained in the initial phase of this study enables assessment of the occurrence and extent of peripheral eosinophilia in strongyloides patients. These data are of interest for two reasons. Firstly, the scientific and laboratory studies that precede this study suggest that eosinophilia may be central to any immuno-metabolic effect that strongyloides infection may have in reducing the prevalence of T2DM[8, 9]. In addition to this, a clinical study that looked at the association between eosinophilia and metabolic disease in China found a negative association[86]. From a clinical point of view, eosinophilia has been suggested as a suitable clinical indicator of strongyloides infection, with varying degrees of reliability reported depending on the setting and the diagnostic tests employed[87, 88, 89].

Helminth infections are known to induce a Th2 weighted immune response which is characterized by increased levels of IL5, producing eosinophilia, high levels of IgE and IgG, and contributing to inflammation and worm killing. It is also recognized however, that in chronic infections helminths are capable of inducing a modified Th2 weighted response, associated with lower levels of IL5, diminished eosinophilia, reduced levels of IgE, a predominance of IgG4. In addition, chronic infection can result in maintenance of alternatively activated macrophages in peripheral tissues via an IL4 and IL13 dependent mechanism, with subsequent effects on insulin sensitivity in peripheral tissues[9, 70]. The sensitivity and specificity of eosinophilia as an indicator of strongyloides infection could therefore be expected to depend upon the chronicity of infection, and could also be expected to reflect the presence or otherwise of insulin resistance and T2DM.

For clinicians practicing in remote locations in Australia, where serological testing may be relatively difficult to obtain, eosinophilia is often used as a "proxy test" for helminth infection. Whether this is a reasonable approach that could be supported by evidence is not known. While studies in migrant and non-endemic populations have produced disparate results, such an analysis has not been attempted in an endemic population in Australia. Data for this paper were collected in 2013 and 2014, and published in 2016.

Hays R, Thompson F, Esterman A, McDermott R, 2016. *Strongyloides stercoralis*, eosinophilia, and type 2 diabetes mellitus: the predictive value of eosinophilia in the diagnosis of s stercoralis infection in an endemic community. Open Forum Infect Dis 3: ofw029.

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4.2 *Strongyloides stercoralis*, Eosinophilia, And Type 2 Diabetes Mellitus: the Predictive Value of Eosinophilia in the Diagnosis of *S. stercoralis* Infection in an Endemic Community

Summary: This paper examines the relationship between *Strongyloides stercoralis* infection and eosinophilia in an endemic community, and suggests that eosinophilia is not a reliable screening test for excluding *S. stercoralis* infection in this setting. The relationship between T2DM, strongyloides infection and eosinophilia is elaborated.

Abstract

Background

This study examines the predictive value of eosinophilia for *Strongyloides stercoralis* infection, as measured by ELISA testing, in an endemic community. In remote communities, eosinophilia is frequently used as a proxy test for the presence of helminth infections. Past studies of eosinophilia and strongyloides infection have been conducted in specific groups such as immigrants and refugees, or in sub-populations of non-endemic communities, rather than in endemic communities.

Methods

We conducted a cross sectional study of the relationship between eosinophilia and strongyloides ELISA serology, as part of a study into the relationship between *S. stercoralis* infection and type 2 diabetes mellitus (T2DM) in an Indigenous community in northern Australia.

Results

Two hundred and thirty-nine adults had their eosinophil count and *S. stercoralis* ELISA serology measured in 2012 and 2013, along with other biometric and metabolic data. Eosinophilia was found to have a relatively poor sensitivity (60.9%), specificity (71.1%), positive predictive value (54.6%), and negative predictive value (76.1%) for *S. stercoralis* ELISA positivity in this group. There was however, a more constant relationship between eosinophilia and *S. stercoralis* ELISA positivity in patients with T2DM (negative predictive value 87.5%).

Conclusion

This study suggests that the presence or absence of eosinophilia is not an adequate proxy test for *S. stercoralis* infection in a community where the infection is prevalent, and that the

association between eosinophilia and *S. stercoralis* ELISA positivity is more constant in patients with T2DM.

4.2.1 *Introduction*

Infection with the soil-transmitted helminth *Strongyloides stercoralis* is endemic in many of the Indigenous communities of northern Australia, with prevalence of up to 41% recorded in some locations.[77]

Direct microbiological tests to diagnose the infection are often impractical in these settings as multiple fresh fecal samples are required, and sensitivity is low even in ideal circumstances. The use of ELISA serology in the diagnosis of strongyloides infection and in conducting prevalence surveys has, in the past, been contentious. Uncertainties exist over the meaning of positive ELISA results, what level of ELISA test should be considered positive, and whether antibodies persevere after the resolution of infection. In addition, cross reactivity with other helminth infections was considered to be a problem, particularly with earlier versions of the test. Recent studies however suggest that the use of an ELISA test to detect antibodies to the worm is both sensitive and specific enough to diagnose the infection and determine the success of treatment, and its use is now widespread in clinical practice.[24, 25, 26] This test however, is currently not available in a point of care format, and therefore entails transport of specimens to central laboratories with significant delays in diagnosis and treatment for patients in remote locations.

Eosinophilia is a common, but not uniform, finding in *S. stercoralis* infection and is thought to be more marked in earlier infections, becoming less pronounced and more variable in chronic cases.[73] Several studies have addressed the relationship between eosinophilia and strongyloides infection in the context of patient screening, but these have been conducted in migrant and traveler populations, or in sub-populations of non-endemic societies [87, 88, 89, 90, 91, 92, 93], and have often not addressed the prevalence of strongyloides in patients without eosinophilia.

Infections that are endemic to northern Australia, and that are known to produce eosinophilia include *Trichuris trichuria*, hookworm species, *Hymenolepis nana, Toxocara canis, Giardia duodenalis* and *Strongyloides stercoralis*[94], as well as ectoparasites such as *Sarcoptes scabiei*, resulting in eosinophilia being a common finding in this region.

As part of a study conducted into the relationship between type 2 diabetes mellitus (T2DM) and *S. stercoralis* infection, we examined the prevalence of eosinophilia in relation to strongyloides infection, as measured by ELISA serology, in an Aboriginal community. Data from this study demonstrated a negative relationship between *S. stercoralis* infection and T2DM[77], and has suggested that T2DM is a predictor of treatment failure in this setting.[95] Eosinophilia is thought to be central to the process by which helminth infections can affect the metabolic status of infected subjects through a process of immunomodulation.[8]

This study provides, for the first time, data on the predictive value of eosinophilia in the diagnosis of *S. stercoralis* infection in an endemic community and analyses this in the context of T2DM.

4.2.2 *Materials and methods*

The study was conducted in three related Indigenous communities, located within a 100km radius in the Tanami desert region of Western Australia. Opportunistic testing for, and treatment of S. *stercoralis* was commenced in these communities in 2012 according to the best practice guidelines of the Australian Strongyloides working group.[31]

S. stercoralis ELISA testing was ordered, often in conjunction with other routine laboratory investigations. Testing was performed by Pathwest Laboratory in Perth Western Australia, using the commercial strongyloides IgG ELISA (DRG laboratory). The reference values, in units of absorbance, for this test were as follows: Less than 0.2 –Negative; 0.2 to 0.4 – Equivocal; Greater than 0.4 –Positive. However, as was noted by the laboratory, these ranges were developed in a metropolitan population where the prevalence of *S. stercoralis* was very low (manufacturers information). In order to reduce the possibility of false negative results it was decided to employ a modified range. All values greater than or equal to 0.30 were considered positive and treated. All values less than 0.30 including those less than 0.20 were considered equivocal and were re-tested after a period of 6 months, in order to ascertain the rate of sero-conversion (and presumably therefore new infections) in this group. Only 3 sero-conversions were found in this time, and the analysis for this study was performed using the results of the initial testing only.

Data was extracted including the age, sex, date of testing, *S. stercoralis* ELISA titre haemoglobin, total eosinophil count, percentage eosinophilia, height, weight, calculated Body Mass Index (BMI), diabetic status and HbA1C Triglyceride level, HDL and total cholesterol.

The study population for this study was identical to that in our two previous studies in this community.[77, 95]

Eosinophilia was defined as a total eosinophil count of 0.50 x10^9/L or greater.

Diabetes was defined in this group as an HbA1C reading of 6.5% or greater, or a random blood glucose of more than 11.1 mmol/l, or a fasting blood glucose of more than 7.0 mmol/l, either at the time of testing, or in the past in patients already receiving treatment for diabetes.

4.2.3 *Ethical approval*

The protocol for this study was approved in principle by the Kimberley Aboriginal Health Planning Forum. All participant were 21 years of age or older. As no investigations or treatments apart from those required for best clinical practice were being performed, and literacy levels are very low in the study population, verbal consent was considered appropriate. Verbal consent was obtained from all participants and recorded electronically. Formal ethical approval was granted by the Western Australian Aboriginal Health Ethics Committee (HREC:515)

4.2.4 *Statistical analysis*

Descriptive statistics, including means, medians, percentages and their respective 95% Confidence Intervals were used to analyze the demographic and clinical characteristics of participants. The accuracy of eosinophilia (≥0.50) as a measure of *S. stercoralis* status (E-titre ≥ .30) was evaluated using sensitivity and specificity measures. To account for the high prevalence of *S. stercoralis*, the Positive Predictive Value (PPV) and Negative Predictive Value (NPV) for the ≥.50 eosinophilia cut off were also calculated. These diagnostic test evaluations were also undertaken separately for participants with and without diabetes.

Four logistic regression analyses were undertaken to examine the association between diabetes status and the sensitivity, specificity, PPV and NPV of eosinophilia as measure of *S. stercoralis*. As a first step, dichotomous variables were created to flag participants that were true positive cases (eosinophilia ≥.50 and E-titre≥.30) or true negative cases (eosinophilia <.50 and E-titre<.30).

The sensitivity regression was the odds of diabetic participants with *S. stercoralis* having a positive eosinophilia diagnosis (true positive) compared to the odds of the same true positive

diagnosis among non-diabetic participants. The specificity regression was the odds of a true negative diagnosis by these diabetes groups.

Analysis of PPV was the odds of a true positive diagnosis among diabetic patients with a positive eosinophilia compared to non-diabetic patients. The NPV regression was the odds of true negative diagnosis among those with a negative eosinophilia result, again by diabetes status.

All regression analyses were also adjusted for sex, age, BMI and previous anthelminthic treatment with albendazole. Cases with missing values on any of these variables were dropped from the adjusted modeling. A 5% significance level was used for all statistical tests and all analyses were undertaken using STATA v13. (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP.)

4.2.5 *Results*

A total of 259 patients were screened, with 92 (35.5%: 95% CI 29.9 – 41.6%) cases of strongyloides infection, as indicated by ELISA serology of ≥ 0.30, diagnosed. Eosinophil counts however, were available for only 239 patients, with the missing values being due to specimen degradation during transport. Of the 20 degraded specimens 5 were positive for *S. stercoralis* by ELISA testing and 7 had T2DM. Table 4.1 details the clinical characteristics of the 239 patients with both ELISA testing results and eosinophil counts.

Table 4.1 Prevalence of Eosinophilia (≥0.5) and *S. stercoralis* (E-titre ≥0.4) by diabetes status.

		***S. stercoralis* serology status (ELISA titre)**					
Diabetes status	**Eosinophils measure**	**Positive (% E-titre ≥0.4)**		**Negative (% E-titre <0.4)**		**Total**	
		n	**%**	**n**	**%**	**N**	**%**
Non-diabetic		**45**	**39.1**	**70**	**60.9**	**115**	**100.0**
	Eosinophilia (≥0.5)	24	53.3	21	46.7	45	100.0
	Non-eosinophilia (<0.5)	21	30.0	49	70.0	70	100.0
Diabetic		**26**	**21.0**	**98**	**79.0**	**124**	**100.0**
	Eosinophilia (≥0.5)	20	38.5	32	61.5	52	100.0
	Non-eosinophilia (<0.5)	6	8.3	66	91.7	72	100.0
Total		**71**	**29.7**	**168**	**70.3**	**239**	**100.0**
	Eosinophilia (≥0.5)	44	45.4	53	54.6	97	100.0
	Non-eosinophilia (<0.5)	27	19.0	115	81.0	142	100.0

Table 4.2 shows that 97 patients had eosinophilia, giving an overall prevalence of 40.6% (95% CI 34.5 – 47.0%). In the infected patients, the prevalence was 60.9% (50.1 – 70.7%). The prevalence of eosinophilia was similar in diabetic compared to non-diabetic subjects, (41.9% (95% CI 33.5 – 50.9%) and 39.1% (95%CI 30.5 – 48.5%) respectively, OR 1.12 95%CI 0.67-1.88, p=0.659) while the prevalence of eosinophilia was higher in infected diabetics compared to infected non-diabetics, although this difference did not reach statistical significance (71.0% 95%CI (51.8 – 84.8%) and 55.4% (95% CI 41.9 – 68.1%) respectively OR 1.97, 95% CI 0.77 – 5.03, p=0.156).

A quarter (25.0%) of the diabetic patients had a positive *S. stercoralis* ELISA test compared to almost half of the non-diabetic patients (48.7%). Eosinophilia as a test for *S. stercoralis* had a sensitivity of 60.9% and a specificity of 71.1% (Table 4.3). Eosinophilia had an overall PPV of 54.6% and a NPV of 76.1%. The NPV of eosinophilia was higher in diabetic patients (87.5%, 95%CI 77.6 - 94.1) compared to non-diabetic patients (64.3%, 95%CI 51.9 – 75.4).

As the ELISA cut off level of 0.30 units was used in this study for purely clinical reasons, the analysis was repeated using the conventional cut off of 0.40 units (Appendix 2 Tables 1-3) and again using a higher cut-off of 0.50 for the purposes of comparison. The NPV of eosinophilia remained high for both cut off levels (81.0% for ≥ 0.40 and 86.6% for ≥ 0.50) and the differences between diabetic and non-diabetic patients remained comparable.

Logistic regression (Table 4.4) showed that among the 97 patients with eosinophilia, the odds of having a positive *S. stercoralis* ELISA test (PPV) were 67% lower among diabetic patients compared to those without diabetes (OR=0.33, 95%CI 0.14–0.77, p=0.010). This difference remained after adjusting for age, sex, weight, BMI and past treatment with anthelminthic drugs (n=95, OR=0.30, 95%CI 0.11–0.81, p=0.018). In comparison, diabetics without eosinophilia were almost four times more likely to also have a negative *S. stercoralis* ELISA test (NPV), compared to non-diabetic patients without eosinophilia (n=142, OR=3.89, 95%CI 1.66–9.12, p=0.002). This difference remained after adjustment, although 14 patients were excluded due to missing values on potential confounding variables (n=128, OR=4.51, 95%CI 1.73–11.76, p=0.002).

Table 4.2 Accuracy of Eosinophilia (≥0.5) as a measure of *S. stercoralis* status determined by serology (E-titre ≥0.4).

Parameters	**Non-diabetic (n=115)**		**Diabetic (n=124)**		**Total (n=239)**	
	n	**(%)**	**n**	**(%)**	**n**	**(%)**
True negatives	49	(42.6)	66	(53.2)	115	(48.1)
False positives	21	(18.3)	32	(25.8)	53	(22.2)
True positives	24	(20.9)	20	(16.1)	44	(18.4)
False negatives	21	(18.3)	6	(4.8)	27	(11.3)
	(%)	**(95% CI)**	**(%)**	**(95% CI)**	**(%)**	**(95% CI)**
Sensitivity	(53.3)	(37.9, 68.3)	(76.9)	(56.4, 91)	(62.0)	(49.7, 73.2)
Specificity	(70.0)	(57.9, 80.4)	(67.3)	(57.1, 76.5)	(68.5)	(60.8, 75.4)
Positive predictive value	(53.3)	(37.9, 68.3)	(38.5)	(25.3, 53)	(45.4)	(35.2, 55.8)
Negative predictive value	(70.0)	(57.9, 80.4)	(91.7)	(82.7, 96.9)	(81.0)	(73.6, 87.1)

Table 4.3 Regression analyses of sensitivity, specificity, positive predictive value and negative predictive value by diabetes status.

Analysis	Diabetes status	Unadjusted odds ratio			Adjusted odds ratio†		
		n	**OR**	**(95% CI)**	**n**	**OR**	**(95% CI)**
Analysis of sensitivity		**71**			**66**		
	Non-diabetic	45	1.00		41	1.00	
	Diabetic	26	2.92	(0.99, 8.62)	25	3.68	(1.05,12.91)*
Analysis of specificity		**168**			**157**		
	Non-diabetic	70	1.00		66	1.00	
	Diabetic	98	0.88	(0.46, 1.72)	91	0.99	(0.47, 2.06)
Analysis of positive predictive value		**97**			**95**		
	Non-diabetic	45	1.00		43	1.00	
	Diabetic	52	0.55	(0.24, 1.23)	52	0.53	(0.20, 1.39)
Analysis of negative predictive value		**142**			**128**		
	Non-diabetic	70	1.00		64	1.00	
	Diabetic	72	4.71	(1.77, 12.56)*	64	5.50	(1.83, 16.56)*

* $p<0.05$
† Adjusted for sex, age, BMI and past antibiotic treatment
Eosinophilia (≥0.5) and *S. stercoralis* (E-titre ≥0.4)

Table 4.4 Logistic regression analyses of sensitivity, specificity, positive predictive value and negative predictive value by diabetes status.

Analysis	Diabetes status	Unadjusted odds ratio			Adjusted odds ratio†		
		n	OR	(95% CI)	n	OR	(95% CI)
Analysis of sensitivity		**87**			**82**		
	Non-diabetic	56	1.00		52	1.00	
	Diabetic	31	1.97	(0.77, 5.03)	30	2.07	(0.73, 5.84)
Analysis of specificity		**152**			**141**		
	Non-diabetic	59	1.00		55	1.00	
	Diabetic	93	0.65	(0.31, 1.37)	86	0.74	(0.33, 1.66)
Analysis of positive predictive value		**97**			**95**		
	Non-diabetic	45	1.00		43	1.00	
	Diabetic	52	0.33	(0.14, 0.77)*	52	0.30	(0.11, 0.81)*
Analysis of negative predictive value		**142**			**128**		
	Non-diabetic	70	1.00		64	1.00	
	Diabetic	72	3.89	(1.66, 9.12)*	64	4.51	(1.73, 11.76)*

* $p<0.05$
† Adjusted for sex, age, BMI and past antibiotic treatment

4.2.6 *Discussion*

This study suggests that eosinophilia alone cannot be used to infer the presence of *Strongyloides* infection in patients from Indigenous communities where the condition is endemic. This is perhaps not a surprising finding given the large number of other parasitic infections that are present in these communities and capable of causing eosinophilia.

More notably however, was the relatively low NPV of eosinophilia, suggesting that the absence of eosinophilia does not reliably rule out the diagnosis of *Strongyloides*

infection in this setting. In fact, in this study almost a quarter of patients without eosinophilia had positive *Strongyloides* ELISA serology. This is of relevance to clinicians because, in a remote setting, the absence of eosinophilia is often used as a proxy test to imply the absence of parasitic infection. This study suggests that such a practice would not be safe as a means to exclude *Strongyloides* infection, particularly in situations where the patient faces immunosuppression or chemotherapy. Immunosuppression and or steroid therapy in the presence of undiagnosed *Strongyloides* infection can lead to hyperinfection syndrome, often with fatal consequences.[22]

The findings are similar to those of Naidu et. al. in their survey of refugee populations in Canada, where they are also at pains to point out that the absence of eosinophilia should not be used to infer the absence of *Strongyloides* infection.[88] They are at odds however with a survey of farm workers carried out in southern Spain where the results suggested that eosinophilia had a specificity of 93.1% and sensitivity of 93.5%, and the authors recommended public health screening for strongyloidiasis using eosinophilia.[93] Clearly the situation in this community is quite different, with a low level of transmission overall and the absence of other significant parasitic infections. This disparity can also be explained in part by differences in the method of diagnosis employed. The Spanish study used microbiological examination of stool specimens for diagnosis, a method that is known to have a low sensitivity, and to be influenced by the worm burden and subsequent numbers of larvae shed in the faeces.[24] Eosinophilia is known to be more common in early or acute *Strongyloides* infections, when worm burden and larval counts are highest, and to decline and become more variable in chronic infection presumably through the mechanism of parasite induced immunomodulation in the host.[13, 73] Use of fecal testing for diagnosis would therefore tend to select earlier infections, with higher worm counts and therefore higher rates of eosinophilia, while ELISA testing would detect more chronic infections where immunomodulation had resulted in more variable eosinophilia.

This study found that the prevalence of eosinophilia in diabetic subjects was no different to that in non-diabetic. This is at odds with the findings of a much larger cross-sectional study performed in China that demonstrated a negative relationship between eosinophilia and insulin resistance and T2DM.[86] The Chinese study,

however, was conducted in a population where both helminth infection and eosinophilia are less prevalent, and where other, non-infectious causes of eosinophilia are likely to be more common.

Data from this community published elsewhere demonstrates a negative relationship between *Strongyloides* infection and T2DM.[77] It is postulated that this effect is again due to parasite-induced immunomodulation affecting the hosts metabolic system in chronic infections and resulting in increased insulin sensitivity. Laboratory evidence suggests that eosinophilic infiltration of adipose tissue due to helminth infection promotes the presence of alternatively activated macrophages, which in turn act to increase insulin sensitivity.[8]

This study however, showed a higher prevalence of eosinophilia in ELISA positive diabetics and demonstrates that the absence of eosinophilia in diabetic subjects is more closely linked with the absence of *Strongyloides* antibodies. This might be explained if the diabetic group contains a greater proportion of acute or recent infections, resulting in a more marked eosinophilia, as opposed to chronic and immune-modulated infections, where eosinophilia is less pronounced and where the past eosinophilic infiltration of adipose tissue has contributed to a lower prevalence of T2DM.

It is equally plausible that T2DM itself is responsible for the higher rate of eosinophilia. A recently published paper looked at the relationship between the human adipokine resistin and multiple helminth infections. It found that higher resistin levels were associated with a more pronounced inflammatory response, a higher worm burden and reduced worm clearance.[56] T2DM along with obesity and metabolic syndrome has been variably linked with elevated levels of resistin.[85] In addition, data from this study published elsewhere found that T2DM was associated with treatment failure in *Strongyloides* infection suggesting that the immune response may be altered in diabetics.[95]

Perceived weaknesses of this study may be that it relies on ELISA serology alone for diagnosis, rather than microscopy which is the most specific test, however we feel this is outweighed by the superior sensitivity of the serological test. Clearly the numbers involved in this study are small, and further larger studies may be of benefit.

In addition, no distinction was made between mild, moderate and severe eosinophilia, with the single cut off point of 0.5 $x10^9$ /l being used. It may be that employing a lower threshold for eosinophilia might improve the negative predictive value of the test.

This study supports the use of ELISA testing for *Strongyloides* infection as a screening test for patients in endemic Aboriginal communities regardless of their eosinophilia status, and suggests the practice of presuming the absence of infection in patients without eosinophilia is not a safe one. It may be that the absence of eosinophilia might be of some use in the decision to screen or treat for strongyloidiasis in the diabetic population in this community.

Further studies would be needed to assess whether this is the case in other settings where the disease is endemic and to further examine the link between eosinophilia and T2DM.

Chapter 5 Outcomes of Treatment for *Strongyloides stercoralis* in an Endemic Community: Control of Infection

5.1 Introduction

Follow up of this cohort of patients has two main objectives. Central to this thesis is the aim of further characterizing the relationship between *S. stercoralis* infection and T2DM, and this will be discussed in Chapter six. As has been noted however, the study design also enables assessment of the long-term effectiveness of ivermectin and its use in a "case finding and treatment" strategy for the control of strongyloides in this community. This is of considerable interest to the many clinicians who are concerned with the control and elimination of *S. stercoralis* infection in Aboriginal Australia, and potentially in endemic communities world-wide. It is also central to the main concerns of this thesis, as attempts to control this infection must inevitably be weighed up against potential adverse metabolic effects in the treated populations.

One of the unique contributions made by this thesis is the duration of follow up that was achieved for this cohort. A time scale of three years was chosen mainly in order to allow the emergence of any effects on metabolic outcomes to become apparent, however, this also enabled a relatively long term follow up of the success of treatment. As stated previously, prior studies had been characterized by relatively short time scales, usually 6 to 12 months, designed to test the efficacy of treatment rather than the effect on disease prevalence, and had often suffered from a high rate of loss to follow up. The protocol for this study was unique in that it employed a "case finding and treatment" approach to the treatment of strongyloides infection, and then examined the outcomes after 3 years for patients who continued to live in an endemic community in the absence of any further attempts at screening or treatment, and in the absence of any environmental control program. It therefore provides evidence as to the effectiveness of such a treatment strategy in sustainably reducing the prevalence of infection in an adult population over the medium to long term. A case finding and treatment strategy stands in contrast to "mass drug administration" (MDA) strategies which have been suggested for affected communities. A recent study published in Australia demonstrated that MDA for strongyloides infection was successful in reducing the prevalence of infection over a limited time period, but did not eliminate

the infection, and had no effect on clinical symptoms[96]. If it could be demonstrated that a case finding and treatment strategy was equally effective in controlling disease prevalence, then the issue of possible adverse metabolic side effects becomes clinically important. A public health measure such as an MDA does not allow for consideration of an individual's metabolic status in the way that a case finding and treatment strategy does. Potential adverse metabolic consequences of treatment, which could be allowed for clinically in a case finding and treatment strategy, could only be balanced by further public health measures in an MDA model.

Collection of the data in this study occurred in 2014 and 2016, and the results were published in 2017

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5.2 Control of Chronic *Strongyloides stercoralis* Infection in an Endemic Community may be Possible by Pharmacological Means Alone: Results of a Three-Year Cohort Study

5.2.1 *Abstract*

Objectives: To assess the effect of treatment with ivermectin on the prevalence of *S. stercoralis* infection in an Australian Aboriginal population over a three-year period, and to assess the validity of using a lower ELISA cut-off in diagnosis.

Methods: A three-year cohort study of 259 adult Australian Aboriginals living in a remote community in northern Australia. *S stercoralis* infection was diagnosed using commercial ELISA testing, and employed a lower threshold for treatment than that recommended. Follow up was conducted at 6 months and 3 years following ivermectin treatment.

Findings: Treatment with ivermectin was highly effective and resulted in a sustained fall in the prevalence of infection in the study group (Initial prevalence 35.3%, 3-year prevalence 5.8%, McNemar's $chi^2 = 56.5$, $p<0.001$). Results of treatment suggested use of a lower ELISA threshold for treatment was valid in this setting. Follow up identified a small group of subjects with persistently positive ELISA serology despite repeated treatment.

Interpretation: Control of *S. stercoralis* infection in this cohort appears to be feasible using pharmacological treatment alone.

5.2.2 *Author summary*

Infection with the worm *Strongyloides stercoralis* is common throughout the developing world, and in some resource poor communities living within developed societies, such as the Aboriginal communities of northern Australia. It is generally agreed that reliable diagnosis of this infection is possible by blood tests, and that the medication ivermectin represents the best available treatment, however questions remain over how to best control and eliminate the infection in areas where it is common.

Strongyloides infections may be asymptomatic, and may persist indefinitely without the need for re-infection. The worm is transmitted by contact with contaminated soil.

Suggested strategies for control have therefore included mass administration of ivermectin in affected areas, and environmental measures to prevent the contamination of soil.

In this study, we follow up a group of subjects living in an endemic community three years after they were tested and treated for strongyloides infection. We find a persisting low prevalence of infection in this group in the absence of any environmental changes or further treatment, suggesting that control of the infection in this community might be achieved through simple case finding and treatment alone. In addition, we suggest the use of a lower cut-off value for serological testing in these communities, in order to avoid missing cases of infection.

5.2.3 *Introduction*

Infection with the soil-transmitted helminth (STH) *Strongyloides stercoralis* is common in both the developing world, and in underprivileged communities in developed countries. Prevalence is frequently estimated at 30- 100 million cases world-wide, but due to difficulties with diagnosis, the frequent absence of symptoms in chronic cases, and the lack of extensive screening for the infection, this may be an under-estimate [23]. The major medical impact of infection is thought to be the occurrence of strongyloides hyperinfection syndrome, which occurs predominantly in infected adults who become immunosuppressed, whether by iatrogenic means or otherwise [22].

It is increasingly acknowledged that *S. stercoralis* infection represents a significant public health challenge and that action is required to deal with the problem [12], however there is no general agreement as to what these measures should be. Some advocate the need for extensive environmental management of the problem to interrupt transmission in endemic communities [18, 97]. They point out that pharmacological treatment can have side effects, cannot prevent re-infection from environmental sources of the infection, and that resistance to ivermectin may develop with excessive use. Others emphasize the efficacy and safety of treatment with ivermectin, and advocate pharmacological control, with possible mass drug administration (MDA) in communities that have a high prevalence of infection in accordance with programs currently employed to control other soil transmitted helminth and vector borne nematode infections [23]. They point out that free-living

forms of the parasite are relatively transient, human beings constitute the major reservoir of infection, and while some resistance has been noted in veterinary settings, to date there is no evidence of resistance in human infection. Still others have advocated a combination of these approaches, employing case finding and treatment with ivermectin, in association with public health measures such as improved sanitation [98].

A measure of success has been reported to date, with studies employing both approaches reporting positive results in control of the infection. [98, 99]

It should be pointed out that the optimal measures for control of strongyloides infection may differ from situation to situation, depending upon local factors such as rainfall and vegetation, the importance of agricultural practices in transmission, and the presence or otherwise of adequate health infrastructure. Because of the chronic nature of this infection and its capacity for sustained auto-infection within individuals, it is possible to have a high prevalence of infection both in communities where conditions allow for high transmission rates of the infection, and equally in communities where transmission is lower, but there are a high number of chronic, untreated infections.

The Aboriginal communities of northern Australia are home to some of the highest measured prevalence of this infection, with rates of 30-40% reported [34].

Oral ivermectin is now accepted as the treatment of choice for this condition, but disagreement persists over the need for wide spread testing and elimination of the infection. Attention has been drawn to some of the barriers preventing control of infection in these communities, including difficulties with diagnosis and lack of adequate follow up of treated cases [76].

Direct parasitological diagnosis of infection and other coprological methods are generally not feasible in these isolated communities, and are known to have a low sensitivity even when performed under optimal conditions. ELISA testing is now generally accepted as being sensitive and specific enough for use in both clinical and research settings [23, 26]. Concerns over cross reactivity with other helminth infections have less relevance in the Australian setting where these infections are not found. In practical terms in the Australian setting this equates to testing using one of several

commercially available ELISA tests. The reference ranges for these tests were developed in relatively low prevalence populations and were intended to prevent false positive results in situations where the infection is uncommon. For example, the unreferenced product information for one such test (DRG laboratories) advises clinicians that normal ranges may need to be interpreted "in the context of local settings". Anecdotally, it is reported that for this reason, clinicians working in communities where the infection is known to be endemic frequently treat patients whose ELISA results fall under the published range, in an attempt to avoid missing infections. To date there have been no attempts to validate this approach, and studies of treatment efficacy have mostly been characterized by limited and poor follow up of treated cases [34].

The current study comprises a three year follow up of a cohort of patients tested and treated for strongyloides infection in an Aboriginal community in northern Australia as part of a study into the relationship between *S. stercoralis* infection and type 2 diabetes mellitus [77]. It is the first study to examine the long-term outcome for adults tested and treated for the infection, who continue to live in an endemic community in the absence of any attempt at environmental manipulation.

Furthermore, as the original cohort was established using a revised (and lower) cut off for ELISA positivity, there can now be an attempt to validate the use of a lower ELISA threshold for treatment by looking at response to treatment and long-term outcomes in these patients.

5.2.4 *Methods*

5.2.4.1 Study population

The study was conducted in three Aboriginal communities located within a 100km radius of each other in the far north Kimberley region of Western Australia. The communities are isolated, with the nearest hospital and laboratory services being 800km away. Prior to the commencement of this study, testing and treatment for *S. stercoralis* infection had not taken place in any systematic manner.

The cohort comprised 259 adult Aboriginals attending the medical facilities in these communities, and was originally established from April 2012 to December 2013 as part of a study into the relationship between *S. stercoralis* infection and type 2

diabetes mellitus (T2DM). The protocol for this study has been published previously[77].

Patients were offered testing and treatment for *S. stercoralis* infection on an opportunistic basis when attending the clinics. No attempt was made to screen patients on the basis of presenting symptoms, as it was felt that there was little evidence for the presence of reliable symptoms in chronic strongyloidiasis in an endemic setting. Recent results from a study conducted in an Aboriginal community would seem to support this [96]. Patients who were normally resident outside the communities were excluded, as were patients who had received prior treatment with ivermectin, without serological testing for *S. stercoralis*.

5.2.5 *Serological testing for S. stercoralis*

Diagnosis of *S. stercoralis* infection was established purely through serological means as direct parasitological studies would have been logistically difficult in this setting, and would likely have lacked sensitivity [25].

ELISA testing was carried out at a reference laboratory utilizing a commercial *S. stercoralis* ELISA kit (DRG laboratories) that tests for the presence of IgG antibodies. All positive results were subsequently tested in parallel and the revised value used in analysis.

The normal ranges (in units of absorbance) provided by the laboratory for this test were

: < 0.20 – Negative
: 0.20- 0.40 – Equivocal
: > 0.40 –Positive

These normal ranges were developed in a low prevalence population however. A prior survey in the study community had suggested that a prevalence of infection greater than 30% was likely [31] and therefore, in order to reduce the possibility of false negative results, a modified normal range was employed for diagnosis. All results greater than 0.30 were considered as “positive”, and were treated. All results less than 0.30, including those less than 0.2, were considered “equivocal” from the point of view of follow up, and were placed on recall for repeat testing in 6 months.

5.2.6 *Treatment and follow up*

All patients returning positive results according to the protocol were treated with 2 doses of ivermectin 0.2mg/kg, given 2 weeks apart under direct observation.

All 259 patients in the study were then recalled after 6 months for repeat serological testing. The results of 6 month testing have been published previously, as part of a study into the relationship of treatment outcomes and T2DM [95].

Any subjects found to be ELISA positive at 6 months were recalled for repeat treatment with a single dose of ivermectin 0.2mg/kg. An audit of patient records shows that of the 29 subjects who remained ELISA positive at 6 months, 24 received an extra dose of ivermectin at this time, while 5 did not.

Routine follow up of patients was then conducted by the individual clinics. Clinic records show that a further 5 subjects received a fourth dose of ivermectin, for presumed strongyloides infection, during the subsequent 2½ years.

All subjects were finally recalled approximately 3 years after initial testing for repeat ELISA testing and metabolic assessment. Any subjects found to be ELISA positive at this time were treated with a further dose of ivermectin 0.2mg/kg. Three year follow up also involved an assessment of the subject's metabolic status as part of an ongoing study into the relationship between strongyloides and T2DM, the results of which will be published separately[100].

5.2.6.1 Treatment outcomes

Treatment outcome in this study was evaluated in two ways. Firstly, "treatment success" was defined as a fall in ELISA serology to < 0.30 at follow up. "Treatment effect" was defined as a ratio of post treatment ELISA to pre-treatment ELISA of less than 0.60.

As a modified normal range was used in this study to identify positive cases, at follow up it was possible to compare two groups; those whose initial serology was above the established positive range of ≥ 0.40 (designated "high titre"), and those whose initial serology was in the range between ≥ 0.30 and< 0.40 (designated "low titre") These

groups were compared in terms of treatment success and treatment effect on the basis that if those in the low titre group represented true infections with *S. stercoralis* (rather than false positives) then treatment outcomes should be similar to those achieved in the high titre group. Additionally, outcomes for the two groups were compared in terms of the average ELISA serology of both groups at 3 years follow up, on the basis that if both groups represented true infections, and both groups were treated in the same way, then the two groups should be similar in terms of outcomes at 3 years

5.2.6.2 Assessment of "non-responders"

During the course of follow up it became apparent that a small number of subjects continued to return positive ELISA results after three years of follow up, despite repeated treatment with ivermectin (designated "non-responders"). Clearly it is of interest as to whether these non-responders represent true treatment failure or re-infection, or whether they are simply continuing to produce a positive ELISA test in the absence of ongoing infection. To aid in the assessment of these subjects, additional testing was carried out, including serological testing for HTLV-1 virus, serum IgE, FBE, and a multivalent faecal PCR test for *S. stercoralis* targeting a 101bp region of the 18S gene (Western Diagnostic Pathology, Myaree) [101]. Molecular testing of faeces specimens was employed, as it is logistically easier to achieve from an isolated location. Studies suggest that, although still less sensitive than serology, it has improved sensitivity over direct parasitological methods[[27]].

5.2.7 *Statistical analysis*

McNemar's chi-square test for correlated proportions was used to compare prevalence rates at the different time points. Comparison of mean age and BMI and change in eosinophil count between responders and non-responders was undertaken using independent samples t-tests. No attempt was made to impute data for those lost to follow up. Logistic regression was used to determine differences in outcome for high titre and low titre cases.

5.2.8 *Ethics statement*

The protocol for this study was approved by the Kimberley Aboriginal Health Planning Forum. Ethical approval for the study was obtained through the Western Australian Aboriginal Health Ethics Committee (WAAHEC) in 2014 (HREC

Reference 515). All subjects were over 18 years of age and provided informed verbal consent. Use of verbal consent was approved by WAAHEC as no additional tests or treatments were required other than those dictated by the current best management guidelines for this condition, and due to variable rates of literacy in the study community. Consent was recorded electronically in the subject's permanent medical record.

5.2.9 *Results*

5.2.9.1 Treatment outcomes

Demographic data for the 259 patients originally enrolled in the study are given in Table 5.1.

Table 5.1 Demographic data.

		Not treated		**Treated**		**Overall**		**Sig.**
		N	**%**	**N**	**%**	**N**	**%**	**P**
Age	<30	18	10.7	17	18.7	35	13.5	0.141
	30-39	64	38.1	26	28.6	90	34.8	
	40-49	44	26.2	20	22.0	64	24.7	
	50+	42	25.0	28	30.8	70	27.0	
	Total	168	100.0	91	100.0	259	100.0	
Sex	Male	69	41.1	37	40.7	106	40.9	0.949
	Female	99	58.9	54	59.3	153	59.1	
	Total	168	100.0	91	100.0	259	100.0	

Successful follow up was achieved in 207 of the original cohort at 3 years, with 52 subjects lost to follow up as outlined in Figure 5.1. Those lost to follow up were similar in terms of infection status (36.5% vs. 35.2%, p=0.85), with a tendency to be female (62% vs. 48%; p=0.071). All other baseline comparisons were similar.

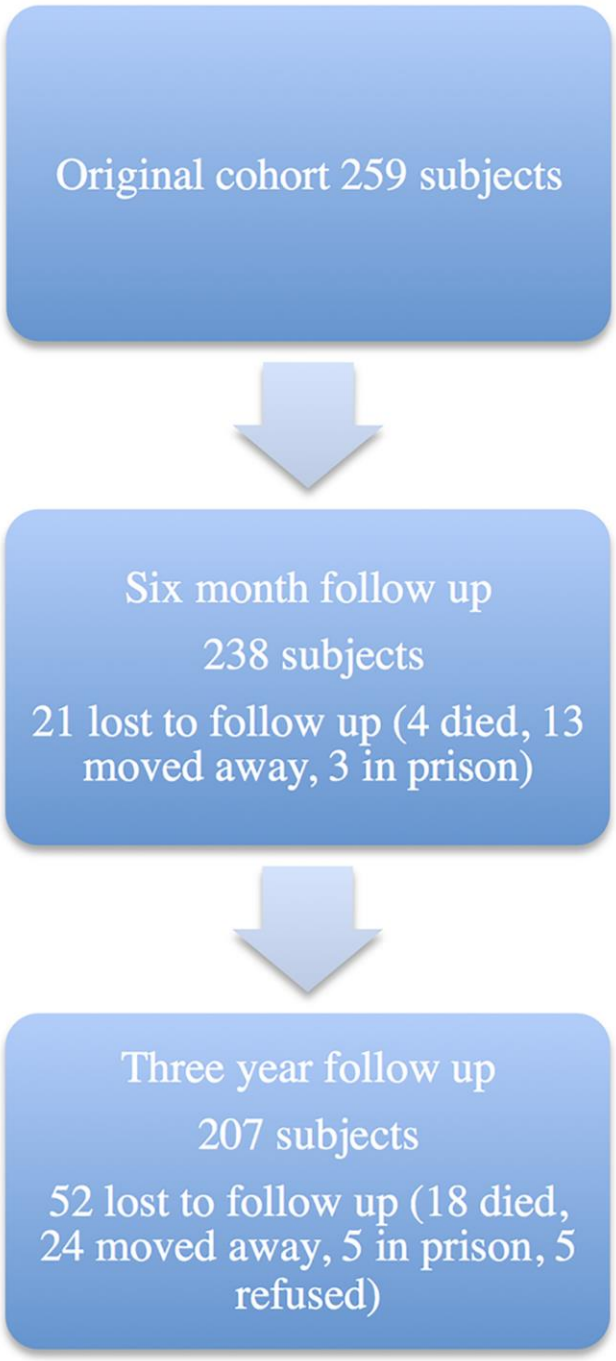
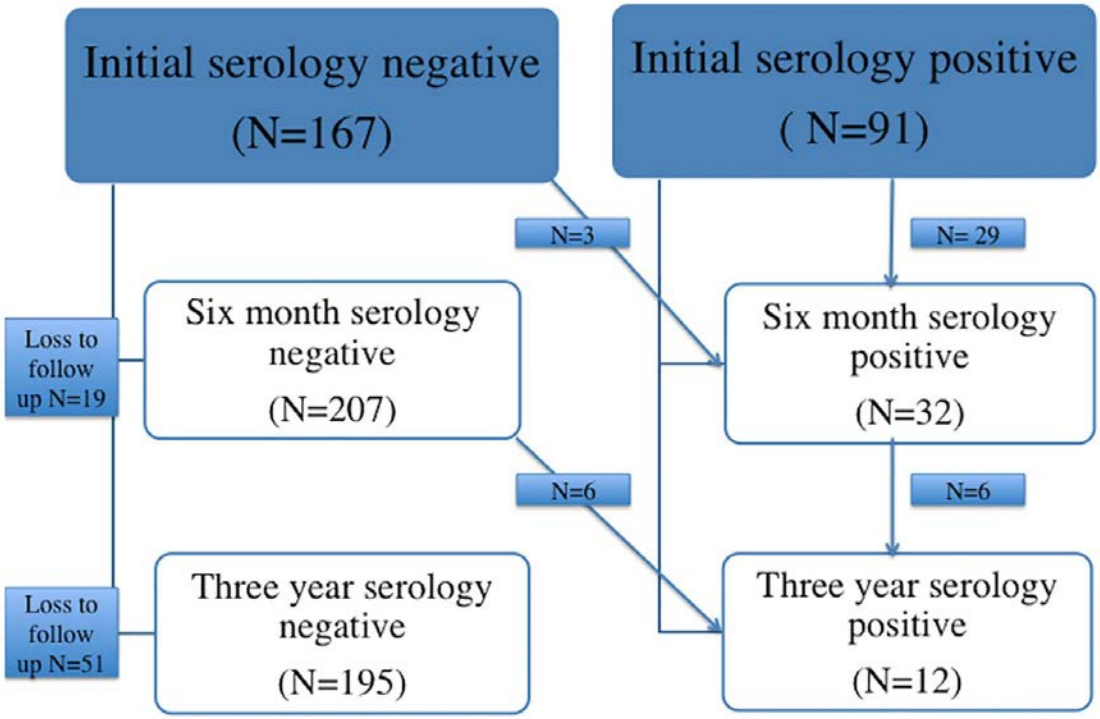


Figure 5.1 Outcome of follow up at three years.

Figure 5.2 gives a graphical illustration of the outcome in terms of serology for those followed up at 6 months and 3 years. Treatment with ivermectin was highly effective, with the prevalence of ELISA positive subjects falling from an initial 35.3% to 13.4% (McNemar's chi2 = 46.6, $p<0.001$) at 6 months and 5.8% (McNemar's chi2 = 56.5, $p<0.001$) at 3 years, respectively.



*One patient who tested positive to *S. stercoralis* but left the community prior to treatment, has been excluded from analysis.

Figure 5.2 Treatment outcome at three years.

Only three new cases were diagnosed in the first 6 months of follow up, with a further three in the period up to 3 years. Three cases that had returned to negative at 6 months were found to be ELISA positive again at 3 years. It should be noted that it is not possible to ascertain whether these three cases represent relapse of existing infection, presumably through the process of "auto-infection" that is known to occur in chronic strongyloidiasis, or re-infection from environmental sources. The details of these subjects are summarized in Table 5.2.

Table 5.2 New cases at follow up.

Subject	Age	Initial ELISA	Six month ELISA	Three year ELISA
Female	53	0.19	0.30^	0.12*
Female	43	0.15	0.43^	0.20*
Female	30	0.18	0.30^	0.22*
Male	43	0.22	-	0.34^
Male	69	0.15	0.24	0.33^
Female	29	0.14	0.03	0.40^
Female	73	0.48^	0.28	0.37^
Female	70	0.51^	0.29	0.37^
Male	43	0.35^	0.27	0.32^

*After treatment at 6 months follow up. - Subject was lost to follow up at 6 months. ^Positive results.

All new cases at 6 months and 3 years involved only a marginal increase in ELISA, with only two cases rising above the accepted positive range of 0.40, and the others deemed positive due to the use of a reduced cut-off in this study. Additionally, the three cases that "relapsed" after cure at 6 months in fact only dipped marginally below the cut-off of 0.30, before returning to marginally above.

An analysis of treatment outcomes at 6 months with respect to treatment failure has been published previously [95].

5.2.9.2 Non-responders

Of the 12 positive cases at 3-year follow up, 9 represent "treatment failure", with these subjects remaining ELISA positive throughout follow up, despite repeated treatment with ivermectin. The details of these subjects are presented in Table 5.3, along with the results of the further tests conducted. Comparisons of mean age, BMI and eosinophil counts with the subjects who responded to treatment are included.

Table 5.3 Details of Non-responders.

	Age	**Initial BMI**	**Initial ELISA (abs.)**	**Six month ELISA**	**Final ELISA**	**Initial eosin.($x10^9$)**	**Final eosin. ($x10^9$)**	**HTLV-1**	**IgE (kU/ml)**	**Fecal PCR**
1 Male	75	30.37	0.65	0.45	0.45	0.59	0.13	Neg	- *	-
2 Female	58	32.94	0.67	0.59	0.4	0.37	0.23	Neg	323	-
3 Female	73	29.6	0.48	0.28	0.37	0.57	0.39	Neg	87	neg
4 Female	70	46.05	0.51	0.29	0.37	0.47	1.06	Neg	-	neg
5 Female	45	29.11	0.59	0.34	0.32	0.36	0.17	Pos	1010	-
6 Male	31	38.85	0.38	0.31	0.32	0.51	0.33	Neg	223	neg
7 Female	40	56.01	0.73	0.58	0.46	0.39	0.42	Neg	137	neg
8 Female	46	34.08	0.35	0.34	0.34	0.94	0.62	Neg	1187	neg
9 Male	43	24.4	0.35	0.27	0.32	0.85	0.62	Neg	214	neg
“Non-responders” average	53.4	35.7					Av. change in eosin. 0.12			
“Responders” average	41.8	28.1					Av. change in eosin. 0.41			
Comparison	Diff 11.7 p =0.024	Diff 7.6 p= 0.005					Diff -0.29 p = 0.17			

*Missing values are indicated by –

Analysis shows them to be significantly older (difference 11.7 years, 95% C.I. 1.6-21.8, p=0.024), and heavier (BMI difference 7.6, 95% C.I. 2.4 -12.9, p= 0.005) than those who responded to treatment, with a smaller fall in eosinophil count after treatment, which did not reach statistical significance (difference -0.29, 95% CI -0.71-0.13, p= 0.167). Four of the seven subjects tested had IgE levels in the normal range.

5.2.10 *Comparison of outcomes for low titre and high titre cases.*

Table 5.4 shows a comparison of the outcomes for the low titre (initial serology ≥ 0.30 and< 0.40) and high titre (initial serology ≥ 0.40) groups.

As can be seen, the difference between the two groups in terms of treatment success is not statistically significant, and they are very similar in terms of average ELISA titre at 3-year follow up. There is a significant difference when compared in terms of "treatment effect", but this not significant when adjusted for initial ELISA titre.

Table 5.4 Outcome for "low titre" subjects.

	"Treatment success" (% success)	**"Treatment response" (% effective)**	**Post treatment average ELISA (95% C.I.)**
"Low titre" ELISA (N=16)	13 (81.2)	10 (62.5)	0.146 (0.08-0.21)
"High titre" ELISA (N=58)	52 (89.7)	54 (93.1)	0.143 (0.11-0.17)
Analysis	O.R. 2.0 (95% C.I 0.44-9.08, p= 0.369	O.R. 8.1 (C.I. 1.9-34.0, p= 0.004) Adjusted O.R.* 2.98(C.I. 0.46-19.31, p= 0.25)	Difference=0.003 (C.I. -0.07-0.06, p= 0.92)

Adjusted for initial ELISA titre

5.2.11 *Discussion*

The results of this study suggest that control [102] of *Strongyloides stercoralis* infection in this adult Aboriginal population could be achieved by case finding and treatment alone. A marked and sustained reduction in infection rates was seen in the study group without the need for environmental manipulation, or repeated follow up and

treatment beyond the current best practice recommendations. The advantage with this strategy is that it requires no change in the current diagnostic or treatment guidelines, simply a sustained clinical response from those agencies providing health care in these communities.

The conditions present in Aboriginal communities differ in several ways from those in populations elsewhere in the world, where different strategies for control of *S. stercoralis* have been employed, and therefore the findings in this study may not be easily generalized.

Rates of *S. stercoralis* infection, as with other soil-transmitted helminths, are thought to rise throughout childhood, peaking in late adolescence or early adulthood, and remaining relatively constant through adult life [14]. In the study community it is likely that most infections occur in childhood and adolescence as a result of recreational activities around watercourses.

A study conducted in Cambodia employed a strategy that involved both case finding and treatment and attempts to improve sanitation, and reported success in control of infection over a two year follow up [98]. The incident rate of new infection was higher in this setting when compared to our study, and transmission was facilitated by agricultural practices that are not a factor in Aboriginal communities. Furthermore, the standard of housing and hygiene facilities in the Cambodian communities was lower and more variable.

It is not the case that improvements in hygiene were not necessary for control of STH infections in Aboriginal communities, rather that most of the necessary changes may have already occurred over the past few decades. Studies of the prevalence of hookworm infection in northern Australia suggest that control has been achieved through both pharmacological means and improvements in housing [39, 40]. Current living conditions in Aboriginal communities remain poor by broader standards, but in the current study community all individuals are, at a minimum, housed in purpose built dwellings with toilet facilities.

Mass administration of ivermectin has been advocated for control of *S. stercoralis* in high prevalence communities. This strategy appears to have been effective in Central American studies examining the administration of ivermectin for control of

onchocerciasis, with sustained falls in the prevalence of *S. stercoralis* infection in areas subject to regular MDA compared to those who are not [99].

Such MDA programs would potentially have secondary health benefits in Aboriginal communities in terms of a reduction in *Sarcoptes scabiei* infections, with a subsequent reduction in Group A streptococcal infection and its sequelae of rheumatic fever and post-streptococcal glomerulonephritis. Trials of this approach have been conducted in Northern Australia with variable results [12]. The results of two MDA's of ivermectin for the treatment of strongyloides in an Australian Aboriginal community have recently been published in this journal. A marked reduction in the prevalence of strongyloides at 6 and 18 months was demonstrated using this approach [96]. Such a strategy however, would require the active participation of affected communities and public health authorities alike, and agreement on the need for such a program has not yet been reached [32].

5.2.11.1 Clinical effects of treatment

The principal aim of this study was to determine the effectiveness of a "case finding and treatment" approach to controlling *S. stercoralis* infection in an Aboriginal community. No attempt was made in our study to characterize the initial symptoms of subjects with chronic strongyloidiasis, and therefore there are no data with regard to improvements in the well-being or health of the community in response to treatment. A recent, much larger trial published in this journal did examine the benefits in terms of symptom control of MDA for strongyloides, and found no evidence of a significant change in symptom profile [96]. Most discussion of the benefits of controlling strongyloides infection has centred on the prevention of hyperinfection in immunocompromised individuals, and such patients are routinely treated on a purely empirical basis in parts of northern Australia [96]. No cases of hyperinfection were recorded in the study community during the course of the study.

Our prior study identified T2DM as a risk factor for failure for treatment of *S. stercoralis* [95]. No meaningful association of T2DM with treatment outcome could be identified at 3 years because of the very low number (9 subjects, 3 of whom had T2DM) failing to seroconvert. Extensive data however have been recorded in regard to the metabolic outcome for the subjects in this study and these will be reported on separately[100]. In summary, the data showed a differential effect of treatment for

S. stercoralis on the metabolic outcomes for patients depending on their diabetic status. The incidence of worsening glucose metabolism in non-diabetic patients (new cases of T2DM or glucose intolerance) was higher in the treatment group (RR 3.75, CI 1.06-13.2, p=0.04). At the same time improving diabetic control as measured by HbA1c was noted in the treated diabetic group (Diff=-1.03, p=0.009). While the low numbers in this trial mean that this result should be interpreted cautiously, they are in agreement with another recently published study into the effect of albendazole treatment for STH infection on subsequent insulin resistance [103]. The possible adverse effect of treatment on glucose metabolism is of importance when considering the case for MDA in strongyloides. As a public health measure, MDA does not allow for consideration of the individual clinical state of the subjects being treated, and therefore intervention to monitor and improve the glucose metabolism of individuals at risk of developing T2DM is not possible.

Limitations of the present study include its reliance on serological means alone for diagnosing *S. stercoralis* infection, as direct parasitological tests are required to ensure maximum specificity. However, as already noted, direct methods lack sensitivity, are difficult to employ in remote locations, and can over-estimate the response to treatment [26].

Several caveats need to be raised on the effectiveness and feasibility of a case finding and treatment strategy. Firstly, the current study deals only with an adult population, and little or nothing is known of the situation with respect to the prevalence of infection, and effectiveness of treatment in the paediatric population. Logically it would seem likely that infection and re-infection rates might be higher in children, where hygiene practices may be less rigorous and activities where infection could occur more commonly. Disagreement on the need for elimination in adults still exists, and this may be even more so in the case of children. Few clear severe adverse health effects of chronic infection have been demonstrated in children, and the major health consequence in adults- the occurrence of strongyloides hyperinfection syndrome in immunosuppressed patients-has been reported only rarely in children [22, 104]. It may therefore be reasonable to employ a regimen where cohorts of children are screened and treated as part of health screening procedures as they reach adulthood. Furthermore, as noted above, there is increasing evidence that pre-existing helminth

infections may have beneficial effects on metabolic parameters in later life, and this potential effect may need to be taken into consideration when considering treatment in populations where type 2 diabetes mellitus is extremely prevalent [105].

Case finding and treatment also relies on the accurate and sensitive diagnosis of infection by the means currently available, as large numbers of false negative tests would be a problem for effective control. The similarity in response to treatment between the "low" and "high "titre groups in this study suggests that it may be appropriate to treat individuals whose ELISA values fall just below the positive range when working in a high prevalence community. Further research into the appropriate ELISA normal range for these populations may be of benefit.

Accurate follow up of treated cases would also be important in a case finding and treatment strategy. A significant number (9 cases, 9.8%) in this study remained ELISA positive throughout the study period despite adequate treatment, and it is important to know whether these represent true infections. Repeated re-infection of these subjects would seem unlikely as an explanation given the low overall incident rate of infection in the population. Resistance of the worm to treatment is certainly a possibility, although this is yet to be reported in a human population [18]. Alternately, it is possible that a persistent antibody response has developed in these individuals in the absence of ongoing infection, in a situation analogous to the "serofast" response sometimes seen in treponemal infection [106]. Further investigation of the 9 subjects in this study has not served to settle the matter. Faecal PCR studies were conducted in 6 cases and were negative, suggesting an absence of infection, or at least an absence of larval shedding at the time of testing. Eosinophilia is known to be an unreliable indicator of infection [107], however it is perhaps of note that eosinophilia rates that differed prior to treatment in the responding and non-responding subjects were almost identical following treatment. HTLV-1 infection is known to pre-dispose to severe and persistent strongyloides infection [33], but was present here in only one individual. IgE levels are commonly elevated in chronic infection but are reported to be normal in some cases, particularly in those co-infected with HTLV-1 and in the elderly [108]. Only two of the patients in this group had elevated levels of IgE at follow up. On balance, the further tests conducted on the 9 non-responder cases in this study do not support the presence of on-going infection with *S. stercoralis*. Clearly further studies

to characterize the immune response in subjects such as these would be of interest, as it is known that changes in relative levels of inflammatory and anti-inflammatory cytokines occur in patients following successful treatment for *S. stercoralis* infection [109].

It should be noted that if the 9 non-responder subjects are excluded from analysis on the presumption that they are no longer infected, then the prevalence of *S. stercoralis* in this cohort 3 years after treatment falls to just 1.4%, underlining once again the success of this treatment strategy.

5.2.12 *Conclusion*

In conclusion, this study provides evidence that control of chronic *S. stercoralis* infection in an adult Aboriginal population may achieved through a process of case finding and treatment, using the current best practice guidelines for treatment. This strategy could be employed as an alternative to mass drug administration, and does not rest upon the need for environmental manipulation.

5.2.13 *Acknowledgments*

The authors wish to formally acknowledge the role of the peoples of the Kutjungka region in this project.

Chapter 6 Outcomes of Treatment for *Strongyloides stercoralis* in an Endemic Community: Metabolic Consequences

6.1 Introduction

The final paper in this thesis documents the follow up of this cohort three years after initial testing and treatment for *S. stercoralis* infection with respect to the metabolic outcomes. The initial cross-sectional study of this cohort had demonstrated a strong negative association between *S. stercoralis* infection and T2DM, but as has been noted, this could not imply causation. The ideal follow-up study, in scientific terms, would therefore presumably be a randomized controlled trial, where some individuals were treated for strongyloides infection, and some were left untreated, with the metabolic consequences being measured subsequently. Such a study would however, fail to meet any ethical criteria. The clinical consequences of *S. strongyloides* are not fully understood, and it is in itself a potentially fatal infection in the context of hyper-infection. Clearly, leaving patients who are known to be infected untreated would be unethical in a setting where treatment is readily available. Despite this, a longitudinal study could go some way towards revealing any effect of treatment if the population of infected and treated individuals could be compared to a group who were uninfected, and therefore untreated. It should be noted a measurable difference in the subsequent prevalence of T2DM and insulin resistance would rely upon the effect of helminth infection on metabolic outcomes being *reversible,* and this is by no means certain. Indeed, evidence from studies into the relationship between past schistosomiasis and T2DM suggest that the effect could be persistent, even for distant infections[47, 48].

The follow up of this cohort also enabled the metabolic outcome for those infected patients with T2DM to be assessed and compared to uninfected individuals with T2DM. Lastly, it enables the follow up of the very small number of patients who remained strongyloides ELISA positive throughout the follow period, despite treatment. These groups may give further insights into the effect of *S. stercoralis* on glucose metabolism.

At the time it commenced, this study constituted the only longitudinal study to examine the metabolic outcome for subjects treated for helminth infection. The

"SUGARSPIN" trial[44], which examined the effect of treatment for soil transmitted helminths on various metabolic parameters, was underway at the same time and ultimately came to publication prior to this paper.

Three years is currently the longest period of follow up achieved in a study such as this. Nevertheless, it remains a relatively brief period of time in the context of the development of insulin resistance and T2DM, and the numbers successfully followed for 3 years is relatively small. Prior studies suggest that the incidence rate of T2DM in northern Australian Aboriginal populations is very high, varying with age from 2.2 per 1000 person-years for those younger than 25 years to 39.9 per 1000 person-years for those 45-54 years[62]. Based on these figures, we expected only 8-12 new cases of T2DM in our cohort over the course of follow up, reducing the likelihood of demonstrating a statistically significant association with treatment. Greater numbers of infected subjects with pre-existing T2DM were available however, allowing meaningful comparisons of treated and untreated subjects with regard to control of T2DM over this time period.

Evidence for a longitudinal effect of treatment for *S. stercoralis* on T2DM and insulin resistance would strengthen the case for a causative relationship between helminth infection and metabolic disease, which has to this time been based solely upon cross sectional observational studies. This would further strengthen the argument for considering metabolic outcomes when planning for the treatment, control and elimination of helminth infections in "transitional" societies.

Acquisition of data for this paper was completed by late 2016, and the results were published in 2017.

Hays R, Giacomin P, Olma L, Esterman A, McDermott R, 2017. The relationship between treatment for *Strongyloides stercoralis* infection and type 2 diabetes mellitus in an Australian Aboriginal population: A three-year cohort study. Diabetes Res Clin Pract. Dec 1;134:8-16.

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6.1.1 *Abstract*

Aim: To determine the effect of treatment for *Strongyloides stercoralis* infection on type 2 diabetes mellitus in an Australian Aboriginal population

Methods: A three-year cohort study of 259 Aboriginal adults living in northern Australia. Subjects were tested for *S. stercoralis* infection, diabetic status and HbA1C at recruitment. 92 subjects were ELISA positive for *S stercoralis* and 91 were treated with two doses of ivermectin 0.2mg/kg. Serological cure was assessed after 6 months and those who remained positive were retreated. All subjects then underwent the same testing at 3 years follow up.

Results: Follow up was successful in 80% of subjects. Eight new cases of T2DM were recorded, 7 in the treatment group and 1 in the non-treatment group (Unadjusted RR 7.71, CI 0.98-60.48 p= 0.052. Adjusted RR 5.45 CI 075-35.92, p=0.093) In addition, worsening glycemic control (T2DM or newly diagnosed glucose intolerance) was recorded in 13 cases (10 treatment group, 3 non-treatment. Adjusted RR 3.74, CI 1.06-13.20, p=0.04,)) There was a significant improvement in glycemic control in the patients with pre-existing T2DM when treated for *S. stercoralis* compared to the non-treatment group (Diff. -1.03, p= 0.009).

Conclusion: This study demonstrated a differential effect of treatment for *S. stercoralis* on glucose metabolism in patients with and without T2DM. It showed a significant effect on the development of T2DM and glucose intolerance in those without T2DM, while improving glycemic control in subjects with pre-existing T2DM. Although numbers in this study are small, it suggests that larger studies may be of interest.

6.1.2 *Introduction*

Recent research has drawn attention to the possible association between helminth infections and both Type 2 diabetes mellitus (T2DM), and metabolic diseases in general, such as insulin resistance, vascular disease and cardiovascular disease. This has been placed in the context of the wider relationship of diabetes to infectious disease, given the rising tide of metabolic illness in the developing world in the setting of persisting high rates of infectious, and in particular, parasitic, diseases[3]. Attention has also been drawn to the possibility that decreasing rates of helminth

infection in the developing world might impact upon the emergence of T2DM and metabolic diseases in these societies[110].

To date, these studies have mostly been retrospective and observational in design, and have demonstrated a protective effect of parasitic infections against metabolic syndrome and T2DM. The infections studied have included Lymphatic filariasis (LF)[46], *Strongyloides stercoralis*[77], Schistosomiasis[47, 48, 54] and soil transmitted helminthes (STH)[49], and have included both past infections (Schistosomiasis) and pre-existing current infections (Strongyloides, LF and STH). Evidence for an effect on other metabolic parameters and cardiovascular disease has to date been variable and less constant[51, 52, 53].

Immuno-metabolic mechanisms, rather than nutritional effects alone, have been proposed to explain the observed relationships. Helminth infections are known to induce anti-inflammatory changes in their hosts through the actions of regulatory T cells and anti-inflammatory cytokines such as IL-10 and TGF−B. [111 112, 113]There is laboratory evidence that helminth infection and subsequent eosinophilic infiltration of adipose tissue can result in metabolic changes that increase insulin sensitivity in peripheral tissues and reduce the likelihood of T2DM[8, 9].

A randomized controlled trial of albendazole treatment for STH infection and its effect on insulin resistance (IR) has recently been reported on in Indonesia[44, 103]. This was a household cluster randomized, placebo-controlled trial involving more than 1500 participants. It was conducted over the course of 12 months, and assessed the effect of administration of albendazole on insulin resistance as measured by HOMA-IR. The study failed to demonstrate an effect at a community level, however it did demonstrate a significant effect on IR in helminth infected individuals receiving treatment, with IR increasing in this group.

The current study reports on the 3-year follow up of a cohort of patients treated for *Strongyloides stercoralis* in an Aboriginal community in northern Australia. The initial study reported in 2015 and demonstrated a strong negative association between pre-existing (current) strongyloides infection and T2DM[77]. Subsequent follow up at 6 months demonstrated that T2DM was a risk factor for treatment failure in this group[95].

The current study examines the rate of development of T2DM and glucose intolerance in the cohort treated for strongyloides infection, and compares it to the rate in the untreated group. In addition, the effect of treatment for strongyloides on glycemic control in patients with T2DM is measured, and the outcome for a small group of patients with a persistent immunological response to *S. stercoralis* is also assessed.

6.1.3 *Materials and methods*

6.1.3.1 Patient cohort

The current study follows up a cohort that was established in 2012/2013 in the course of a cross sectional, observational study into the relationship between *Strongyloides stercoralis* infection and T2DM[77]. The patient group was derived from adult patients attending a clinic in a remote Aboriginal community in the Kimberley region of northern Australia. Subjects were recruited in an opportunistic manner from the group of adults over the age of 20 years attending the community clinic for routine medical treatment, and who consented to being tested and treated for *S. stercoralis* worm infection. A process of community consultation prior to the study ensured that the study was well accepted. Patients who did decline testing had this recorded in their medical record but had no further data recorded for the study.

Patients who had been previously treated with ivermectin without testing for *S. stercoralis* were excluded from the initial study. Where patients were deceased during the course of the follow up period they were excluded from analysis, and efforts were made to determine the cause of death. Subjects who had permanently moved away from the study community during the course of the follow up period were also excluded. A small number of subjects who were in the original cohort refused further follow up and they were also excluded.

6.1.3.2 Parasitological testing

Patients were tested for *Strongyloides stercoralis* infection utilizing a commercial IgG ELISA test (DRG laboratory), and had other biometric, metabolic and biochemical data recorded at presentation, including BMI, HbA1C and full blood examination. All patients with an ELISA titre greater than or equal to 0.30 were treated with two doses of ivermectin 0.2mg/kg /dose and the entire cohort was followed up after 6 months to determine both the effects of treatment on the infected group, and the incidence of new infections in the untreated group. The results of this follow up were reported in

2015. Full details of these two study protocols and of the study community have been published previously[77, 95]. The current study follows up the same cohort approximately 3 years after initial testing, using the same protocol. During the course of follow up it became apparent that a small number of patients had remained ELISA positive for *S. stercoralis* throughout the study, despite often repeated treatment (defined as "non-responders"). In order to better characterize this group, additional investigations were performed at the conclusion of the study period to determine whether the patients remained infected with strongyloides, and the nature of the immune response that was occurring (see below). Fecal specimens were taken to test for *S. stercoralis* DNA and other STH infections, using a multivalent PCR test developed at Western Diagnostic pathology in Perth, Western Australia.

6.1.3.3 Metabolic testing

Diabetes status was determined on the basis of HbA1c, or random blood glucose levels (BGL), and/or treatment status, with T2DM defined as an HbA1c of ≥ 6.5%(48 mmol/mol) or a random BGL of >11.2 mmol/l, or any patient currently receiving treatment for T2DM on the basis of past assessments as outlined above. Additionally, impaired glucose tolerance (IGT) was defined as any patient with an HbA1c ≥ 6.0%(42 mmol/mol) but < 6.5%[114] in the absence of T2DM therapy. Investigations that rely upon a fasted status (fasting BGL or glucose tolerance testing) are rarely performed in the clinics where this study was undertaken due to the transient nature of the population and their cultural practices, and they were therefore not included as part of this study. All testing was performed by an Australian government accredited commercial laboratory (Pathwest Laboratory, Perth W.A.)

For patients taking treatment for T2DM, a treatment adherence measurement tool was devised specifically for this study. This involved calculating the number of weekly packs of prescribed medicine that the patient collected from the clinic in the 12 weeks prior to their final HbA1c measurement, and thereby designating each patient with a score between 0 and 12.

6.1.3.4 Immunological parameters

Additional blood specimens were taken from the "non-responder" group, to measure baseline levels of IL-10, IL-6 and TNF-α, and for whole blood stimulation studies to determine the capacity of the patient's white cells to produce these cytokines.

All subjects were clinically well at the time of examination. In particular they showed no signs of fever or infection, were not using steroids, and had no known relevant medical conditions, other than T2DM or glucose intolerance. Comparison samples were also taken from a group of subjects who were initially positive for strongyloides infection, remained positive at 6 months follow up, but ultimately returned negative samples at 3 years (defined as "responders"). Specimens were packed in ice and transported to James Cook University, Cairns, Queensland where analysis was commenced about 96 hours after the blood was drawn. Mononuclear cells were isolated using density gradient separation methods according to the manufacturer's instructions (Lymphoprep TM), and then stimulated with media or LPS (20ug/ml), and analyzed for production capacity of the cytokines TNF-α, IL-6 and IL-10 after incubation, using a commercial ELISA platform (affymetrix eBioscience).

6.1.4 *Statistical methods*

Descriptive statistics included mean, medians (where data was not normally distributed) and percentages, and their respective 95% confidence intervals (CI). Comparisons of variables between the treated and untreated groups used independent samples t-tests and Mann–Whitney U-tests for continuous variables, and Chi-square tests for categorical variables. 95% CI for medians was obtained by bootstrapping. The association between treatment history and subsequent diabetes status was assessed using a log binomial generalized linear model. An adjusted model was performed using age, sex, baseline HbA1c and change in BMI. Finally, the calculations were repeated excluding those for whom treatment had been unsuccessful.

A similar process was undertaken comparing treatment history and change in HbA1C for patients who were initially in the diabetes group. In this case multivariable linear regression was used, and adjustment was also undertaken for initial HbA1C and for adherence to prescribed medication.

Finally, an analysis of the "non–responding" group was performed utilizing independent samples t-tests for comparison with the responding group in regard to age, sex, eosinophilia, HbA1c and change in HbA1c. Cytokine levels were analyzed using mixed effects models. All analyses were undertaken using STATA v13

(StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP).

6.1.5 *Regulatory commitments*

The protocol for this study was approved by the Kimberley Aboriginal Health Planning Forum. Ethical approval for the study was obtained through the Western Australian Aboriginal Health Ethics Committee (HREC Reference 515).

Further approval for the cytokine studies was sought and obtained in 2016. Patients undergoing these tests were counseled, and provided additional written consent.

6.1.5.1 Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sector.

6.1.6 *Results*

Follow-up outcomes and baseline demographic and metabolic data are recorded in

Figure 6.1 and Table 6.1.

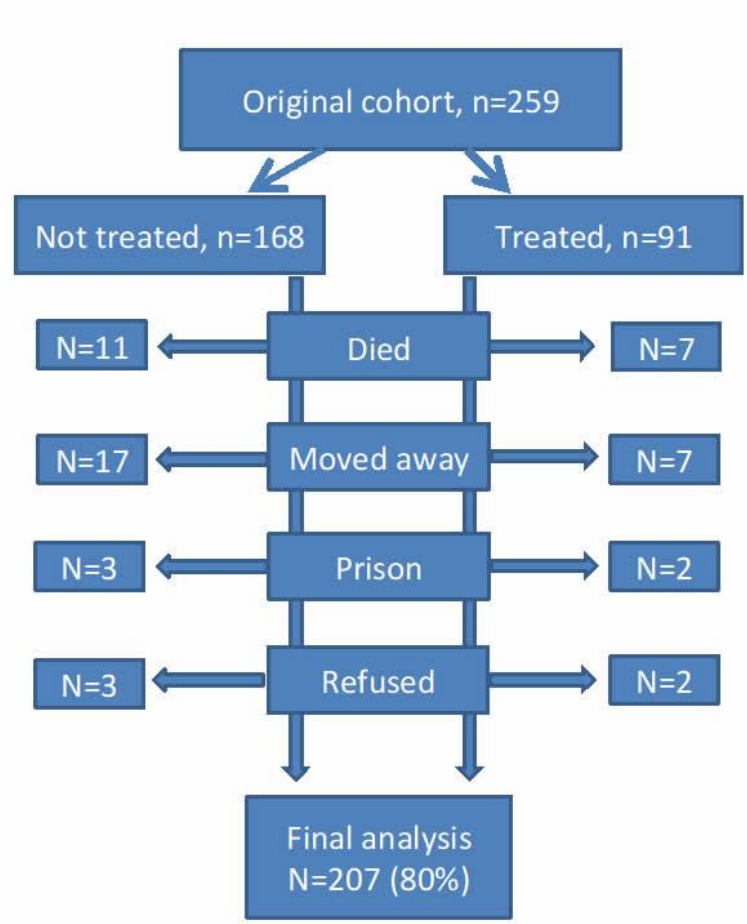


Figure 6.1 Follow-up outcomes at 3 years.

Table 6.1 Baseline Demographic and metabolic data by treatment group.

			Not treated		Treated		Overall	Sig.
		N	**%**	**N**	**%**	**N**	**%**	**P**
Age	<30	18	10.7	17	18.7	35	13.5	0.141
	30-39	64	38.1	26	28.6	90	34.8	
	40-49	44	26.2	20	22.0	64	24.7	
	50+	42	25.0	28	30.8	70	27.0	
	Total	168	100.0	91	100.0	259	100.0	
Sex	Male	69	41.1	37	40.7	106	40.9	0.949
	Female	99	58.9	54	59.3	153	59.1	
	Total	168	100.0	91	100.0	259	100.0	
Glucose metabolism	Normal	54	32.2	40	43.9	94	36.3	<0.001
	IGT	13	7.7	19	20.9	32	12.4	
	T2DM	101	60.1	32	35.2	133	51.3	
	Total	168	100.0	91	100.0	259	100.0	
Tests		**N**	**Mean(SD)**	**N**	**Mean(SD)**	**N**	**Mean(SD)**	
BMI		158	29.9 (7.2)	88	29.3 (8.5)	246	29.7 (7.7)	0.543
HbA1c	%	161	7.6 (2.1)	85	7.0 (1.9)	246	7.3 (2.0)	0.041
	(mmol/mol)		(59.6)		(53)		(56.3)	
Random BGL	mmol	162	9.7 (5.4)	89	8.7 (4.8)	251	9.4 (5.2)	0.148

Follow up was successful in 207(79.9%) of subjects. Of those lost to follow up, 19 were from the treatment group and 33 from the untreated group (OR 1.06, CI 0.56-1.99, p= 0.86). The largest group lost to follow up was those shifting to other communities or in the prison system. Eighteen subjects died during the follow up period, comprising 7 from the treatment group and 11 from the untreated group (OR 1.18, CI 0.44- 3.16, p=0.74). Cause of death, where known, was determined from the medical records, and comprised cardiovascular disease (7), malignancy (2), respiratory disease (2), sepsis (2), and chronic renal failure (1).

Of note are the markedly different baseline tests of glucose metabolism between the infected (treated) and uninfected (untreated) groups (Chi-squared <0.001). This difference in the prevalence of T2DM was the subject of our first paper regarding this cohort[77]. When considering outcomes at 3-year follow up, clearly the proportion in each group who began with IGT is of relevance. However, when considering the effect of IGT on outcome it is not the raw baseline data that is of relevance, rather the proportion in each group with IGT, of those patients *without diabetes* who completed follow-up at 3 years. (From Table 6.2 and Table 6.3, N=207. 18/59 (30.5%) with IGT for the treated group and 12/54(22.2%) for the untreated group, Chi-squared=0.984 on 1 d.f, P=0.321.) Thus, while there is a trend for IGT to be more common in the treated group, this does not reach statistical significance. This trend is allowed for in our adjusted GLM by introducing a term for baseline HbA1c that reflects initial IGT status (see Table 6.4, Table 6.5, and Table 6.6).

Table 6.2 and Table 6.3 show metabolic parameters at baseline and three-year follow up according to treatment group.

Table 6.2 Comparison of metabolic parameters for subjects without T2DM at baseline and follow up (N=95). NT =not treated T =treated

	NT group	**N**	**Mean**	**. SD**	**T grp N**	**Mean**	**SD**	Sig.
BMI	Baseline	50	28.50	6.86	42	27.96	8.04	0.731
	Final	50	29.89	6.79	40	28.77	8.25	0.483
	Change#	47	0.95	3.78	40	0.63	2.20	0.636
HbA1c (%) (mmol/mol)	Baseline	48	5.77 (39.6)	0.30	40	5.80 (39.9)	0.32	0.738
	Final	51	5.65 (38.3)	0.31	40	5.72 (39.0)	0.24	0.222
	Change#	46	-0.12	0.28	38	-0.08	0.22	0.525
Random BGL (mmol/l)	Baseline	49	5.81	1.48	40	6.33	2.15	0.179
	Final	46	6.57	1.88	40	7.12	1.84	0.179
	Change#	44	0.73	21.9	38	0.77	2.94	0.949

*Based on independent samples t-test
#Final – Baseline. Missing values excluded
N=95. Discrepancies in numbers for individual measures are due to missing values

Table 6.3 Comparison of metabolic parameters for subjects with T2DM at baseline or follow up (N=112). NT= not treated T =treated

	NT group	N	Mean	SD	T group N	Mean	SD	Sig.
BMI	Baseline	77	30.85	6.72	31	30.46	7.23	0.789
	Final	78	30.14	6.43	32	30.67	7.50	0.709
	Change#	76	-0.75	2.70	31	0.15	2.85	0.125
HbA1c (%) (mmol/mol)	Baseline	80	8.39 (68.2)	1.78	31	8.55 (69.9)	2.21	0.697
	Final	80	8.84 (73.1)	2.31	32	8.25 (66.7)	2.14	0.214
	Change#	80	0.45	1.58	31	-0.32	1.88	0.032
Random BGL (mmol/l)	Baseline	79	12.07	5.79	32	11.42	5.44	0.585
	Final	80	13.56	6.29	30	13.10	6.21	0.733
	Change#	70	1.57	6.60	30	1.63	7.23	0.971

*Based on independent samples t-test
#Final – Baseline. Missing values excluded
N=112. Discrepancies in numbers for individual measures are due to missing values

Table 6.2 and Table 6.3 show the metabolic parameters for the patients with and without diabetes by treatment group at recruitment and after three years. There are no significant differences between treated and untreated subjects in the baseline groups apart from a significant difference in the change in HbA1c between the treated and untreated diabetic groups, that is discussed in more detail below.

In particular it should be noted that there is no significant difference in BMI between the treated and untreated groups, either at baseline or follow up, and that the change in BMI is also statistically similar between these two groups.

6.1.6.1 Follow up of patients without T2DM

Table 6.4 shows the outcome three years after treatment for patients who were initially classified "no diabetes" (normal and IGT), and demonstrates an increased risk of developing diabetes in the treated group.

Table 6.4 Newly developed diabetes at three-year follow-up.

Treatment status	**No diabetes (%) (normal & IGT)**	**Diabetes* (%)**	**Total**
Untreated	53 (98.2)	1 (1.8)	54
Treated	42(85.7)	7(14.3)	49
Total	95	8	103
	Chi^2= 5.54	P= 0.019	
Unadjusted log binomial GLM	RR =7.71	95% CI 0.98-60.48	P =0.052
Adjusted log binomial GLM**	RR= 5.45	95% CI 0.75-39.52	P= 0.093

GLM: generalized linear model RR: Rate ratio
* Those with HbA1C≥ 6.5% at 3-year follow-up. Three of the new cases in the Treated group developed from subjects with normal baseline HbA1c. The remainder had IGT.
** Adjusted for age, sex, baseline HbA1c and change in BMI

This trend is also present in the group moving from normal HbA1c to impaired glucose tolerance (Table 6.5), although this does not achieve statistical significance.

Table 6.5 Newly developed glucose intolerance at three-year follow up.

Treatment status	Normal (%)	IGT* (%)	Total
Untreated	40 (95.2)	2 (4.8)	42
Treated	25 (89.3)	3 (10.7)	28
Total	65	5	70
	Chi2= 0.89	P= 0.34	
Unadjusted log binomial GLM	RR =2.25	95% CI 0.40-12.62	P =0.36
Adjusted log binomial GLM**	RR= 2.47	95% CI 0.52-11.64	P= 0.25

GLM: generalized linear model RR: Rate ratio
N=70. Three patients in the treated group who were "normal" at baseline developed T2DM at 3 years
* Impaired glucose tolerance. Those with HbA1c≥ 6.0 but< 6.5 at 3-year follow up
** Adjusted for age, sex, baseline HbA1c and change in BMI

When the two groups are combined, the trend for worsening glucose metabolism in the treated group is once again evident (Table 6.6).

Table 6.6 Worsening glucose metabolism at three-year follow up.

Treatment status	No worsening (%)	Worsening* (%)	Total
Untreated	51 (94.4)	3 (5.6)	55
Treated	39 (79.6)	10 (20.4)	49
Total	90	13	103
	Chi2= 5.14	P= 0.023	
Unadjusted log binomial GLM	RR =3.67	95% CI 1.07-12.58	P =0.038
Adjusted robust poisson regression**	RR= 3.74	95% CI 1.06-13.20	P= 0.040

RR: Rate ratio
* Those with HbA1c≥ 6.5%, or newly diagnosed glucose intolerance at 3-year follow up
** Adjusted for age, sex, baseline HbA1c and change in BMI

6.1.6.2 Follow up of patients with T2DM

Table 6.7 demonstrates the outcome in terms of HbA1c for the patients who had T2DM at the initial assessment.

Table 6.7 Effect of treatment on HbA1c in patients with diabetes over 3 years.

Group	**Number**	**Mean change in HbA1c %**	**Coefficient of change score**	
Untreated	79	0.43 (C.I. 0.08- 0.79)		
Treated	25	-0.60 (C.I. -1.41- 0.22)		
Linear regression			-1.03 (C.I. -1.80- -0.26)	P=0.009
Adjusted for baseline*			-0.94 (C.I. -1.71- -0.16)	P=0.019
Adjusted linear regression**			-1.00 (C.I. -1.84- -0.15)	P=0.021

* Adjustment was made for initial HbA1C on the basis that a fall in HbA1C would be more likely from a high starting point
** Adjusted for age, sex, change in BMI, initial HbA1C and adherence to prescribed medication.

A clear improvement in HbA1c over three years is demonstrated for those who were treated for a positive *strongyloides* ELISA. This improvement persists when corrected for initial HbA1c, change in BMI and compliance with prescribed treatment.

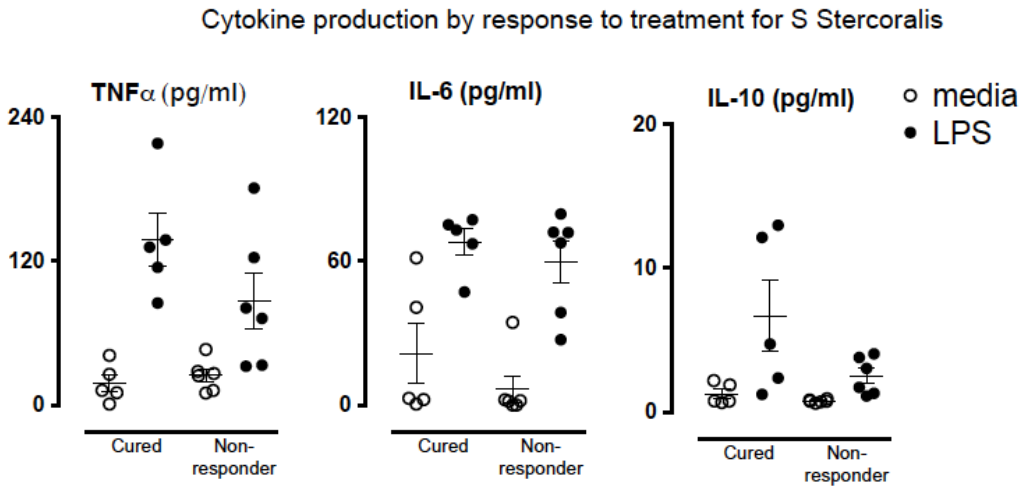
6.1.7 *Follow up of "non-responders"*

Table 6.8 shows demographic and treatment outcome data for the small group (9 patients) who failed to respond serologically to treatment, and remained ELISA positive after 3 years, despite repeated treatment. Where appropriate, comparisons with the remainder of the treated group who responded to treatment (N=82) are included. The non-responder group was older (difference 11.7 years, CI 1.5-21.8, p=0.01)), and heavier (BMI difference 7.6, CI 2.4-12.9, p= 0.002) than the group who responded to treatment, and tended to have a lower initial *Strongyloides* ELISA and lower eosinophil count, although these results did not achieve statistical significance. Most notably, this group demonstrated a marked improvement in glycemic control, as measured by HbA1c, when compared to the group who responded to treatment (Change in HbA1c difference 1.20, CI 0.34-2.06, p=0.007)).

Table 6.8 Treatment after 3 years. “Non-responders” compared to “Responders”.

	Age	Initial ELISA	Initial HbA1C % (mmol/mol)	Initial BMI	Final ELISA	Final HbA1C % (mmol/mol)	Final BMI	Change in HbA1C%
1 Male *	75	0.65	10.6 (88)	30.37	0.45	6.4 (46)	30.1	-2.2
2 Female	58	0.67	6.1 (43)	32.94	0.4	6.1 (43)	34.9	0
3 Female	73	0.48	6.3 (45)	29.6	0.37	5.9 (41)	31	-0.4
4 Female	70	0.51	6.1 (43)	46.05	0.37	5.9 (41)	46.2	-0.2
5 Female *	45	0.59	9.8 (84)	29.11	0.32	6.1 (43)	28	-3.7
6 Male	31	0.38	6.1 (43)	38.85	0.32	6.1 (43)	34	0
7 Female	40	0.73	6.1 (43)	56.01	0.46	5.9 (41)	55	-0.2
8 Female	46	0.35	12.8 (116)	34.08	0.34	10.5 (91)	34.4	-2.3
9 Male	43	0.35	5.6 (38)	24.4	0.32	5.5 (37)	24.6	-0.1
Average	53.4		7.7 (61)	35.7		6.5 (48)	35.4	-1.23
Responders	41.8		6.9 (52)	28.1		6.9 (52)	28.8	-0.03
Comparison	Diff 11.7 p=0.024		Diff 0.83 % p= 0.25	Diff 7.6 p= 0.005		Diff -0.40 % p= 0.55		-1.20 % p= 0.007

*These patients required cessation of treatment for T2DM during the course of follow up due to persistently low blood glucose levels
*** Fecal PCR testing was performed on 6 of the 9 subjects and was negative in all cases



Statistical analysis

TNF-α	Media	Diff 6.46, CI -6.7- 19.62, p=0.337
	LPS	Diff -50.38, CI -98.36- -2.4, p=0.04
IL-6	Media	Diff -14.86, CI -33.76- 4.04, p=0.123
	LPS	Diff -4.23, CI -12.69- 4.22, p=0.33
IL-10	Media	Diff -0.48, CI -0.92- -0.052, p=0.03
	LPS	Diff -4.18, CI -7.56- -0.80, p=0.015

Figure 6.2 Cytokine production by response to treatment for *Strongyloides stercoralis.*

This figure charts the cytokine production values obtained for six of the "non-responder" patients in comparison to a group who responded to treatment. There are differences between the "non-responders" and "responders" when it comes to levels of TNF–α and IL-10 produced on stimulation with LPS, with the group that shows a persistent antibody response to *S. stercoralis* having lower levels of cytokine production on crude analysis.

6.1.8 *Discussion*

The results of this study and their statistical significance clearly needs to be interpreted in the light of the small numbers involved and the differing baseline metabolic measures of the groups compared. As noted above, pre-existing IGT tends to be commoner in the treatment group, although this does not reach statistical significance. Having said this, the current study gives support to the hypothesis that pre-existing infection with the helminth *Strongyloides stercoralis* can have a beneficial effect on glucose metabolism in humans. When taken together the study

findings demonstrate a differential effect of treatment for *S. stercoralis* infection on metabolic outcomes dependent on the diabetes status of the subjects. The results of our study also support the recent findings by Tahapary et al who found that treatment of STH infected individuals with albendazole was associated with an increase in insulin resistance after 12 months.[103]

The current study is the longest duration of follow-up to demonstrate an association between treatment for helminth infection and worsening glucose metabolism and it does so in three ways.

Firstly, follow up of subjects without diabetes at the commencement of the study showed that those who were treated for strongyloides infection had an unadjusted 7.7 times greater chance of being diagnosed with T2DM in the 3 years following treatment than an uninfected and untreated group. This relationship persisted when adjusted for age, sex and change in BMI and initial HbA1c, although the strength of the relationship was reduced (RR 5.85 C.I. 0.75-39.52, p= 0.093) and it failed to achieve statistical significance. Furthermore, patients without T2DM had a tendency to develop impaired glucose tolerance at a higher rate than the untreated group. When both these groups are considered together- that is to say as "worsening glucose metabolism", the relationship persists and is stronger (Adjusted RR 3.74, C.I. 1.06-13.20, p= 0.04)

A cogent theory to explain this observation can be developed from what is already known about the effect of helminth infections on insulin sensitivity. Wu et al (2010)[8] demonstrated that eosinophilic infiltration of adipose tissue was capable of inducing the production of alternatively activated macrophages which then enhanced insulin sensitivity in peripheral tissues via means of the production of anti-inflammatory cytokines such as IL-10 and TGF-B, and the concomitant down-regulation of pro-inflammatory cytokines. It is feasible therefore, that removing or reducing this effect through treatment of a pre-existing strongyloides infection, could worsen insulin sensitivity in a susceptible individual and result in worsening glucose metabolism.

This study also examined the outcome in terms of HbA1c control for patients with T2DM who were treated for strongyloides infection, in comparison to the cohort who were untreated. A strong treatment benefit was demonstrated with an average

difference in HbA1c of -1.03 in the treated group. This benefit persisted when corrected for age, sex and change in BMI. It also persisted when allowance was made for adherence to prescribed medicines. The effect was ameliorated when adjusted for initial HbA1c, on the presumption that it is easier to achieve a large fall in HbA1c if beginning from a high value.

There are several possible explanations for this observation. Firstly, the beneficial effect of pre-existing strongyloides infection may be persistent despite treatment of the infection, resulting in an improvement in glycemic control over 3 years. This would seem to be in keeping with some of the existing observational studies of the relationship between infections such as schistosomiasis and T2DM, as these studies demonstrated an effect purely on the basis of past, rather than ongoing, infections[47, 48, 54]. It is also feasible that strongyloides infection in patients with existing T2DM resulted in worsening of diabetes control, and that treatment subsequently improved the situation. The infected diabetic group had higher HbA1c on average at the outset and there was a higher eosinophil count in infected patients with T2DM[107]. It is plausible that the group with diabetes therefore had a more pronounced Th2 weighted immune reaction with higher levels of Th2 inflammatory cytokines, rather than a modified Th2 response with high levels of the anti-inflammatory cytokines IL-10 and TGF-B as discussed above. Treatment of the infection could therefore have reduced the intensity of the inflammatory reaction and resulted in improved glycemic control. A recent study by de Ruiter et al has demonstrated a differential effect of treatment for STH infection on measures of the TH2 weighted immune response, reporting a reduction in levels of Ig E, but a tendency to increased levels of IL-5 in patients treated with albendazole. They conclude that treatment for helminth infection may have differential effects on TH2 mediated responses, that need to be taken into account when considering the impact of "de-worming" treatment on non-communicable diseases.[115]

Lastly, support is given to the hypothesis by examining the small group of patients who failed to respond to ivermectin treatment and remained ELISA positive to *S. stercoralis* throughout the course of the study. Results of fecal testing suggest that these patients no longer had an active *S. stercoralis* infection. Whether the persistence of antibodies in this group represents true treatment failure, or whether it simply

reflects ongoing antibody production in the absence actual infection, it does suggest that at least some of the immunological changes caused by strongyloides infection persisted in this group of patients for the duration of the study.

Examining the characteristics of this group, they are on average older and heavier, yet despite this they showed a marked improvement in HbA1c over the course of the study in comparison to the successfully treated group. Examining them case-by-case, all had stable or falling HbA1c over the study period, and of the three subjects withT2DM, two had their HbA1c fall into the "non-diabetic" range despite having their diabetes medication ceased during this period.

The cytokine studies performed on this group involve only a very limited number of subjects, and were intended therefore as a hypothesis generating exercise. Past studies have characterized the changes in serum cytokine levels that occur after the treatment of otherwise well individuals[109]. Our study emulates that performed by Wiria et al[49] which failed to find any change in cytokine levels following treatment for STH infection. Despite its limited scope, our study demonstrates a significant difference between community members with successfully treated past infection and those with persisting positive ELISA to *S. stercoralis*, and suggests that persisting ELISA positivity to *S. stercoralis* may have some effect in reducing cytokine production. Adequately powered studies comparing infected individuals, treated individuals and suitable controls may therefore be of benefit.

This study has several limitations. Firstly, the numbers involved are very small; only 8 new cases of T2DM were diagnosed during the course of the study, and the follow up period of three years is relatively short in the context of a chronic metabolic disease like T2DM. Further, the tests used to diagnose T2DM and glucose intolerance are purely clinical ones and therefore relatively insensitive for assessing glucose metabolism. Five of the eight new cases of T2DM had an HbA1c in the range of 6.0%- 6.5% at the commencement of the study, meaning that relatively small changes in HbA1c resulted in a new diagnosis.

Due to logistical and ethical limitations, when performing the cytokine measurements in this study we were unable to test untreated patients and were not able to obtain control specimens from uninfected subjects in the study community.

Lastly, the status of patients involved in the study, with regard to infection and treatment, was necessarily not blinded to either the investigators or the subjects, introducing the possibility of treatment bias. This was mitigated somewhat by the fact that the ongoing care of patients in this clinic was a collaborative effort between several clinicians, and the existence and nature of the study being performed was not disclosed to those involved in the day to day management of T2DM.

Despite these shortcomings, a statistically relevant relationship has been demonstrated between treatment for *S. stercoralis* infection and subsequent glucose metabolism, suggesting that further studies with larger numbers of patients and a longer time frame may be of interest.

6.1.9 *Acknowledgments*

The authors wish to thank the peoples of the Kutjungka region for their participation in this study

Chapter 7 *Strongyloides stercoralis* and T2DM: Conclusions and New Directions

The five papers that constitute this thesis present findings that can be divided broadly into two categories. Firstly, there are findings which speak directly to the hypothesis that helminth infection, in this case with *Strongyloides stercoralis*, has an impact on the development and treatment of type 2 diabetes mellitus. Secondly, the papers provide insights which impact on the diagnosis, treatment and control of *Strongyloides stercoralis* infection, particularly in the Australian context. In this conclusion, I wish to consider both of these areas separately but pay particular concern to the situations where the two elements impact upon each other. I will then consider what practical impact the results might have on research in both the field of T2DM control and treatment, and the control of *Strongyloides stercoralis* infection in the Australian context.

7.1 Conclusions: Metabolic Outcomes

The results delivered in this thesis support the hypothesis that chronic helminth infection can impact upon glucose metabolism in human beings, and that an immuno-metabolic explanation for this finding is most plausible. They do so in the following ways.

Firstly, the initial cross sectional observational study demonstrates a strong negative association between pre-existing *S. stercoralis* infection and a diagnosis of T2DM in an Aboriginal population. Crucially this association persists even after correction for factors such as age, sex and BMI, which suggests that a purely nutritional explanation for the findings may not be valid. Whilst in a study of this kind causation can clearly not be implied, the question of a plausible mechanism for the relationship falls heavily in favour of the infection resulting in a lower prevalence of T2DM, rather than the converse.

In order to conclude instead that T2DM results in a lower prevalence of strongyloides infection, we would need evidence of a plausible mechanism. Candidates for such a mechanism would include the prospect that hyperglycaemia can protect against the establishment of chronic helminth infection, that T2DM patients receive preferential

treatment for strongyloides infection or that this treatment is more effective, or that patients with T2DM and impaired glucose metabolism behave in a way which protects against the acquisition of worm infection. In none of these cases is there any evidence to support these hypotheses, and in the case of treatment effectiveness, there is evidence to the contrary[95]. On the other hand, laboratory evidence into the effects of experimental worm infections on metabolism has provided direct evidence that helminth infection can protect against T2DM, and has provided a plausible immuno-metabolic pathway for such an effect[8, 9].

Secondly, this thesis provides evidence that T2DM impacts upon the short-term success of treatment for *S. stercoralis* infection. Again, several theories could explain this observation, including reduced absorption of ivermectin in diabetic patients, drug interactions with medications commonly prescribed for T2DM, and the possibility that patients with T2DM have a higher proportion of recent infections, and therefore a higher worm burden, with a correspondingly higher rate of treatment failure. Once again, evidence to support any of these hypotheses is lacking. An immune-metabolic explanation can once again be developed, as the inflammatory environment in patients with T2DM is known to differ greatly from those with normal glucose metabolism, suggesting that worm killing and clearance could therefore be affected. Little in the way of experimental evidence exists to support this theory, although one paper has pointed to a role for raised levels of the adipokine resistin and reduced worm clearance in helminth infections[56].

Lastly, the follow up of this cohort three years after treatment provides evidence that treatment for *S. stercoralis* infection may result in worsening of glucose metabolism when compared to an untreated and uninfected cohort. This is evident in the increased prevalence of "worsening glucose metabolism" in previously non-diabetic subjects, and is further supported by the limited cytokine studies, and follow up of "non-responding" patients.

This is one of only two studies to examine prospectively the metabolic outcomes of treatment for helminth infection[103], and although the numbers are small and the follow up period relatively brief, the results suggest that an effect is present, and that larger and more extensive studies may be of interest. Significantly, the results also suggest that the successful treatment of *S. stercoralis* infection may have a beneficial effect on

glycaemic control in patients with pre-existing T2DM. This is not at odds with the other findings of the study, rather it emphasizes the very different immunological and inflammatory conditions which pertain in diabetic patients when compared to non-diabetics, and the relationship that this has to the immunological reaction to helminth infection. Our results are consistent with a more prolonged and intense inflammatory reaction in patients with T2DM and *S. stercoralis* infection, when compared to non-diabetics, and it is therefore conceivable that treating the infection in diabetics may reduce the inflammation, and thus improve diabetic control.

The possibility that treatment for *Strongyloides stercoralis*, or indeed any helminth infection, might worsen the impact of T2DM on either an individual or public health level, is clearly of great concern. The emergence of T2DM and metabolic illness in regions where helminth infection is common has generally been attributed to the dietary and lifestyle changes which are an inevitable consequence of development. The prospect of a reduction in the prevalence of helminth infection in itself also contributing to this problem now also needs to be taken into consideration.

The situation in the Aboriginal communities of northern Australia is at the extreme end of this problem, with T2DM present at epidemic levels, resulting in enormous morbidity and mortality, and affecting progressively younger cohorts of patients[4]. The prospect of worsening this situation through the treatment of strongyloides, which in itself has an uncertain disease impact at a population level, is clearly a concern. A more pertinent question however, might be not to ask why the presence of pre-existing helminth infection prevents T2DM in some individuals, but to ask why T2DM and insulin resistance is occurring in such large numbers in these populations of relatively young people. If we accept the hypothesis that long term immunomodulation by helminths producing a persistent modified Th2 immune response can protect against T2DM, then it would seem reasonable to hypothesise that a persistent Th1 weight immune activation in response to bacterial and viral infection may pre-dispose to the development of metabolic illness in the same group[9]. Despite advances in public health and housing, Aboriginal children and young adults living in remote communities grow up and live in a sea of chronic and acute bacterial and viral infection, suffering from conditions such as chronic suppurative ear disease, streptococcal skin infection, pneumonia, dental sepsis, gingivitis, and venereal

diseases in extremely high numbers. For many therefore, the years from birth to maturity are characterized by an almost constant activation of their Th1 weighted immune response, with possible consequent effects on their glucose metabolism.

In addition to the possible direct effects of persistent infection on metabolic outcomes, the effects of treatment for these conditions also needs to be considered. The frequent and widespread use of broad spectrum antibiotics for actual and presumed infection is almost certainly having an effect on the intestinal flora and "gut microbiota" of Aboriginal children. Alterations in the gut microbiota are known to be associated with T2DM and insulin resistance[41]. Prompt medical treatment for common infections with a range of antibiotic agents is now universally available in Aboriginal communities, but the decision to treat is frequently delivered through protocols administered by nursing staff, whose approach is understandably influenced by the high prevalence of disease, and the consequences of non-treatment of conditions such as otitis media and streptococcal infection on future morbidity and mortality. While clearly a reduction in the frequency of these infections is of primary importance, measures which can improve the precision of diagnosis and effectiveness of treatment, and thereby reduce the unnecessary administration of antibiotics could also be of benefit.

Recognition of the role of chronic inflammation in the pathogenesis of T2DM, and the capabilities of helminths to reduce inflammation, raises the possibility that novel agents for the treatment and prevention of T2DM may be developed through further research in this field

In recent years there has been increased interest in the role of anti-inflammatory agents in the treatment of T2DM and its complications, and clinical trials using inhibitors of cytokines such as TNF-α and IL-1B, and less specific anti-inflammatory agent such as salicase, have been conducted with varying results[74, 116].

Helminths are known to produce their immunological effects through the production of excretory-secretory proteins which have potent effects on the host immune system and the nature and effects of a number of these substances have been documented[75, 117]. Furthermore, it has been established that low level clinical exposure to some helminth infections such as *T. suis* and hookworm is safe. A number of clinical trials have been conducted into the effect of exposure to worm infections and to the

excretory-secretory proteins helminth infections produce, on inflammatory and atopic illness such as inflammatory bowel disease, asthma and atopic eczema[118]. To date there have been no studies employing this approach with metabolic disease.

7.2 Conclusions: Control of *S. stercoralis* Infection

Aside from its findings on the metabolic effects of helminth infection, this thesis also provides insights into the epidemiology, diagnosis and management of *S. stercoralis* infection in Australia. A clear picture of the extent and prevalence of strongyloides infection in Australia is still lacking, and the data in this thesis add to the picture being developed by current projects to improve the extent and quality of information[12].

Results of treatment follow up after 6 months demonstrate that ivermectin is an effective treatment for strongyloides infection in this community, and are compatible with results achieved elsewhere in the world, reinforcing the role of ivermectin as the treatment of choice for this condition.

In the field of diagnostics, this thesis provides further evidence that IgG ELISA testing is valid for the diagnosis and follow up of strongyloides infection. Furthermore, it provides evidence that the use of a lower cut-off point for ELISA testing in high prevalence communities may be valid, and that more extensive studies in this area may be of use.

The data with regard to eosinophilia and strongyloides infection demonstrates that eosinophilia is not a reliable proxy test for strongyloides infection in high prevalence settings, and where other causes of eosinophilia are also prevalent. The presence of higher and more constant rates of eosinophilia in T2DM patients when compared to those without diabetes, once again demonstrates evidence of a differential immune reaction. The higher rate of eosinophilia found in diabetic patients suggests a more robust Th2 weighted immune reaction and a relative absence of immunomodulation in these cases, giving further support to the immuno-metabolic model.

Lastly, the three-year follow up data demonstrates that a “case finding and treatment” model for the diagnosis and treatment of strongyloides has been highly effective in reducing the prevalence of infection in adults living in this community.

These findings, along with the core finding that *S. strongyloides* infection has an effect on the prevalence and outcome of T2DM, has clear implications for strategies being considered for the control and elimination of this infection in Australia and internationally. Broadly speaking, the current strategies available include mass drug administration in endemic communities, environmental manipulations to reduce exposure to the worms, and the case finding and treatment approach employed in this thesis.

Mass drug administration has been shown to have significant effects on the prevalence of infection in regions where it has been employed, both as a consequence of programs to eliminate onchocerciasis in Central America[99], in association with environmental measures in Cambodia[98], and in dedicated programs conducted in Northern Australian Aboriginal communities[96]. It should be noted that while reductions in prevalence have been achieved through MDA, there is as yet no evidence that this can lead to interruption of transmission or elimination of the infection.

Environmental manipulations such as improved hygiene have been beneficial in the context of treatment programs, and circumstantially would seem to be responsible for the long-term control and eradication of the infection in the developed world. Recent studies into the possible role of dogs as a reservoir for some human infections raise the possibility that this may also be important in control programs[17].

MDA raises difficulties of its own, such as community acceptance, sustainability, and most tellingly, the possibility of adverse and unforeseen consequences. Balanced against this is the uncertain disease burden produced by chronic *S. stercoralis* infection, but the very real risk of catastrophic hyper-infection that exists, particularly in immunocompromised individuals. The possibility, and now the evidence, that treatment for strongyloides may produce adverse metabolic consequences is clearly a factor to be considered in any MDA program, particularly in communities where T2DM and its effects are already responsible for such a heavy disease burden. If public health measures such as MDA were to have a negative impact on T2DM on a community level, then this could only be countered by other community wide public health measures to combat T2DM. In contrast, a case finding and treatment approach enables the individual circumstances and metabolic profile of each person to be taken

into account when treatment and follow-up options are being considered. The effectiveness of such an approach in reducing the prevalence of infection in the adult population demonstrated in this thesis, suggests that such a strategy could be successfully employed. While case finding and treatment has not been demonstrated to be effective in preventing transmission in children, or in eliminating the infection from a population, its ability to reduce the prevalence of infection in the population most at risk of hyper-infection may reduce the disease impact of this infection to negligible levels, while limiting any adverse metabolic effects. This approach could be seen as analogous to the "burden of disease" reduction strategies employed by the World Health Organization in the control of other STH infections, where the aim is not to eliminate the worm infection from a population, rather to reduce the burden of infection in those people most susceptible to the disease effects of the worm[119].

7.3 Further Research

The findings in this thesis suggest several avenues for further research. Firstly, the prospective findings of an increase in the incidence of T2DM and worsening of glucose metabolism following treatment for *S. stercoralis* infection demand further investigation through larger multi-centre trials conducted over a larger time span. Randomized controlled trials in this area are difficult to conceive of as they would necessarily fall short of ethical approval. Cohort studies identifying children and teenagers with *S. stercoralis* infection, and then following their metabolic profile into adulthood could be of significant interest, both in determining the potential long-term effects of childhood helminth infection on metabolism, and in comparing the outcomes for treated individuals compared to those who are found to have persisting infection in adulthood. The success of a case finding and treatment strategy in controlling the infection in a paediatric population, and in reducing transmission of the infection would also be of great interest.

A population of particular interest is pregnant women. Strongyloides infection in pregnancy presents unique challenges, as ivermectin is not currently licenced for use in pregnancy, and effective treatment is therefore not available until after delivery. Routine screening for strongyloides infection as a part of antenatal care is not universally adopted throughout northern Australia. Studies which examine the potential effect of ongoing infection on the rate of gestational diabetes are likely to be

of interest. Strongyloides also poses a significant potential risk to pregnant women through iatrogenic hyper-infection, and an ongoing danger to the babies of infected mothers through possible vertical and neonatal transmission. The development of protocols for dealing with the presence of strongyloides infection in pregnancy and the immediate post-partum period should be developed as a matter of priority[120].

Finally, laboratory investigations to isolate some of the candidate excretory/ secretory proteins produced by *S. stercoralis* infection, to determine the safety of their use in humans, and to determine their possible immuno-metabolic effects, could hold out the long-term prospect of identifying novel chemotherapeutic agents for the prevention and treatment of T2DM in susceptible populations. This thesis makes a contribution by beginning to unravel the relationship between one of the oldest infections known to man, and one of the most challenging illnesses to confront health services in the coming decades.

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Appendix 1 Summary of Search

Databases Searched: Medline: 1980 – November 2017, Embase: 1980 – November 2017.

- English language or foreign language articles with an English abstract were searched.
- Only human studies were included.
- All study designs or publication types (research articles including clinical trials, systematic reviews, review articles, editorials, letters, case reports) were included.
- Includes conference abstracts in Embase.

Summary of Search:

The association between helminth infections (i.e. helminth, nematode, cestoda, trematode, soil transmitted helminths, hookworm, trichuris, ascaris, strongyloides including Strongyloides stercoralis, schistosoma, filariasis, onchocerca and opisthorchis) and metabolic diseases (i.e. metabolic disease, type 2 diabetes mellitus, insulin resistance, glucose intolerance, atherogenic index, atherosclerosis, vascular disease, HOMA-IR, resistin, adiponectin and leptin)

The search strategy was devised using two main concepts: (1) 'Helminth infections' AND (2) 'Metabolic disease'. The specific terms searched for each of these concepts are outlined in the 'Summary of Search' section above. Some of these search terms were kept broad such as 'metabolic disease' and 'vascular disease'. All the search terms were majored to make them the main focus of the papers in order to minimise the number of irrelevant references retrieved. Both major thesaurus terms (located in the MeSH/Subject field of references) and text words were identified for each concept. With regard to 'helminth infections', the thesaurus term was exploded to retrieve narrower more specific infections such as 'strongyloidiasis'. 768 references were retrieved using this search strategy and titles were manually scanned for relevancy. A total of 67 references were identified, and the abstracts were accessed. The references of review articles were in turn reviewed. This resulted in a total of 38

relevant publications (15 research articles, 18 review articles, 1 systematic review and 4 others). These are listed below.

1. Nunes M, Guimaraes-Junior MH, Diamantino AC, Gelape CL, Ferrari TCA, 2017. Re: Cardioprotective manifestations of chronic helminth infections: new aspects of an old disease. Heart 103: 1651-1652.

2. Abdoli A, Rasti S, 2017. Cardioprotective manifestations of chronic helminth infections: new aspects of an old disease. Heart 103: 1651.

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Appendix 2 Tables

Appendix Table 1 Prevalence of Eosinophilia (≥0.5) and *S. stercoralis* (E-titre ≥0.4) by diabetes status

Diabetes status	Eosinophils measure	***S. stercoralis* serology status (ELISA titre)**					
		Positive (% E-titre ≥0.4)		**Negative (% E-titre <0.4)**		**Total**	
		n	**%**	**n**	**%**	**N**	**%**
Non-diabetic		**45**	**39.1**	**70**	**60.9**	**115**	**100.0**
	Eosinophilia (≥0.5)	24	53.3	21	46.7	45	100.0
	Non-eosinophilia (<0.5)	21	30.0	49	70.0	70	100.0
Diabetic		**26**	**21.0**	**98**	**79.0**	**124**	**100.0**
	Eosinophilia (≥0.5)	20	38.5	32	61.5	52	100.0
	Non-eosinophilia (<0.5)	6	8.3	66	91.7	72	100.0
Total		**71**	**29.7**	**168**	**70.3**	**239**	**100.0**
	Eosinophilia (≥0.5)	44	45.4	53	54.6	97	100.0
	Non-eosinophilia (<0.5)	27	19.0	115	81.0	142	100.0

Appendix Table 2 Accuracy of Eosinophilia (≥0.5) as a measure of *S. stercoralis* status determined by serology (E-titre ≥0.4)

Parameters	**Non-diabetic (n=115)**		**Diabetic (n=124)**		**Total (n=239)**	
	n	**(%)**	**n**	**(%)**	**n**	**(%)**
True negatives	49	(42.6)	66	(53.2)	115	(48.1)
False positives	21	(18.3)	32	(25.8)	53	(22.2)
True positives	24	(20.9)	20	(16.1)	44	(18.4)
False negatives	21	(18.3)	6	(4.8)	27	(11.3)
	(%)	**(95% CI)**	**(%)**	**(95% CI)**	**(%)**	**(95% CI)**
Sensitivity	(53.3)	(37.9, 68.3)	(76.9)	(56.4, 91)	(62.0)	(49.7, 73.2)
Specificity	(70.0)	(57.9, 80.4)	(67.3)	(57.1, 76.5)	(68.5)	(60.8, 75.4)
Positive predictive value	(53.3)	(37.9, 68.3)	(38.5)	(25.3, 53)	(45.4)	(35.2, 55.8)
Negative predictive value	(70.0)	(57.9, 80.4)	(91.7)	(82.7, 96.9)	(81.0)	(73.6, 87.1)

Appendix Table 3 Regression analyses of sensitivity, specificity, positive predictive value and negative predictive value by diabetes status

Analysis	Diabetes status	Unadjusted odds ratio			Adjusted odds ratio†		
		n	OR	(95% CI)	n	OR	(95% CI)
Analysis of sensitivity		**71**			**66**		
	Non-diabetic	45	1.00		41	1.00	
	Diabetic	26	2.92	(0.99, 8.62)	25	3.68	(1.05, 12.91)*
Analysis of specificity		**168**			**157**		
	Non-diabetic	70	1.00		66	1.00	
	Diabetic	98	0.88	(0.46, 1.72)	91	0.99	(0.47, 2.06)
Analysis of positive predictive value		**97**			**95**		
	Non-diabetic	45	1.00		43	1.00	
	Diabetic	52	0.55	(0.24, 1.23)	52	0.53	(0.20, 1.39)
Analysis of negative predictive value		**142**			**128**		
	Non-diabetic	70	1.00		64	1.00	
	Diabetic	72	4.71	(1.77, 12.56)*	64	5.50	(1.83, 16.56)*

* $p<0.05$

† Adjusted for sex, age, BMI and past antibiotic treatment
Eosinophilia (≥0.5) and *S. stercoralis* (E-titre ≥0.4)

Appendix 3 Published Papers

Appendix 3A: Hays R, Esterman A, Giacomin P, Loukas A, McDermott R, 2015. Does *Strongyloides stercoralis* infection protect against type 2 diabetes in humans? Evidence from Australian Aboriginal adults. Diabetes Res Clin Pract 107: 355-61.

https://files.acrobat.com/a/preview/81667bc3-81d8-4d15-8d6c-c9598b231b12

Appendix 3B: Hays R, Esterman A, McDermott R, 2015. Type 2 diabetes mellitus is associated with *Strongyloides stercoralis* treatment failure in Australian Aboriginals. PLoS Negl Trop Dis 9: e0003976.

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Appendix 3C: Hays R, Thompson F, Esterman A, McDermott R, 2016. *Strongyloides stercoralis*, eosinophilia, and type 2 diabetes mellitus: The predictive value of eosinophilia in the diagnosis of *S stercoralis* infection in an endemic community. Open Forum Infect Dis 3: ofw029.

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Appendix 3D: Hays R, Esterman A, McDermott R, 2017. Control of chronic *Strongyloides stercoralis* infection in an endemic community may be possible by pharmacological means alone: Results of a three-year cohort study. PLoS Negl Trop Dis 11: e0005825.

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Appendix 3E: Hays R, Giacomin P, Olma L, Esterman A, McDermott R, 2017. The relationship between treatment for *Strongyloides stercoralis* infection and type 2 diabetes mellitus in an Australian Aboriginal population: a three-year cohort study. Diabetes Res Clin Pract. Dec 1;134:8-16.

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