LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

Mycobacterium tuberculosis is the agent that causes tuberculosis, a disease that is estimated to infect about one third of the world's population (WHO, 2005). 29% of the global burden of tuberculosis is in sub-Saharan Africa where the incidence rate of smear positive tuberculosis in 2005 was 350 cases per 100 000 population (WHO, 2005).

Childhood tuberculosis contributes significantly to the global burden of this disease, with about one million children developing tuberculosis annually worldwide, which accounts for about 11% of all cases of tuberculosis (Marais et al. 2006; Fincham, 2001).

Childhood tuberculosis differs from adult tuberculosis by the way in which the immune system responds. Children under five years of age are more likely than adults to develop tuberculosis disease following infection and the time from infection to disease is likely to be shorter in children than in adults (Walls et al. 2004). This will have implications for the prevention of progression from infection to tuberculosis disease (Marais et al. 2006; Fincham, 2001).

Tuberculosis is contagious and spreads through the air. When a person with active pulmonary tuberculosis coughs, *Mycobacterium tuberculosis* is spread into the immediate environment in microdroplets which can remain in the air for prolonged periods. People in contact with an active case of pulmonary tuberculosis may inhale these micro droplets containing *Mycobacterium tuberculosis* (Elias et al. 2006; Grosset, 2003; Campbell et al. 2006). After infection with tuberculosis the infected person mounts a response to the pathogen which is so effective that on average 90% of humans with an immunocompetent system are able to control or clear the infection and avoid progression to tuberculosis disease (Hanekom et al. 2007; Elias el al. 2001; Grosset, 2003).

The variation in the ability of the human body to handle tuberculosis has been explored (Elias et al. 2001). Various suggestions regarding the difference in protection against tuberculosis disease have included exposure to environmental mycobacteria (atypical mycobacteria), nutritional differences, genetic or physiological differences and perturbation of the immune system by chronic infectious diseases including helminth infection.

Specifically, chronic helminth infection can result in functional impairment of the immune response that is necessary to clear or control infection by *Mycobacterium tuberculosis.* Protection against tuberculosis is associated with a predominant T helper lymphocyte Type 1 (Th1) cell mediated immune response to mycobacterial antigens, characterised by interferon-gamma release, while susceptibility to tuberculosis is associated with a reduced Th1 response. Helminth infection results in a depressed Th1 cell mediated response, and Th2 polarisation, with or without an elevated T regulatory lymphocyte (Treg) response, which results in a reduction of interferon-gamma production necessary for protection against *Mycobacterium tuberculosis* (Elias et al. 2001; Rook et al. 2006; Fincham 2001).

Helminth infection could thus have a marked effect on the human response to *Mycobacterium tuberculosis* and facilitate the spread of this disease (Bundy et al. 2000; Elliot et al. 2007; The Lancet Editorial, 2004).

Communities with a high prevalence of helminth infection are often those where the incidence of tuberculosis is high – typically those where the living conditions are poor and where over-crowding, poverty and malnutrition are common (Elias el al. 2001; Campbell et al. 2006). It is estimated that more than two billion people worldwide have helminth infections. In endemic areas, children could be infected as soon as they are able to crawl. (Elias et al. 2001). In a study conducted in the Eastern Cape and KwaZulu-Natal, about 20% of children aged less than one year were infected with helminths (Smuts et al. 2004).

There is convincing evidence that helminth control in the Western Cape is neglected (Bentwitch et al. 1999). Established benefits of community control of helminth infection include enhancing development in children, reduction in anaemia and reduction in malnutrition (Alderman et al. 2006).

In addition to enhancing the nutritional status, growth and intellectual development of children (WHO, 2005), there is accumulating evidence that prevention of helminthiasis by mass deworming could improve the immune response to tuberculosis infection and thus potentially decrease susceptibility to tuberculosis and slow progression to disease once infected (Adams et al. 2005; Marais et al. 2006; Fincham, 2001; Bundy et al. 2000; The Lancet Editorial, 2004).

It is thus possible that introduction of regular, six-monthly deworming of children under the age of five years and the inclusion of mass deworming in immunisation campaigns could result in a reduction of helminth infections in this age group, and result further in a reduction in tuberculosis disease.

Between 26th March 2001 and 31st July 2006, a randomised controlled trial was conducted by the South African Tuberculosis Vaccine Initiative which compared two methods of BCG vaccination administration in preventing tuberculosis in very young children.

The primary objective of the trial was to compare the efficacy of the percutaneous to that of the intradermal route of administering BCG in preventing tuberculosis during the first two years of life. The primary endpoint of the trial was the incidence rate of tuberculosis during the first two years of life.

The study was based in the Boland Overberg region of the Western Cape in the Republic of South Africa, centred at Worcester, where the burden of tuberculosis is high. Smear positive detection rate of tuberculosis in this area

was 602/100 000 in 2004 (Nicol et al. 2008). The population has a relatively low HIV sero-prevalence for South Africa: between 5.7 and 6.2% of pregnant women attending public health service antenatal clinics are HIV sero-positive (Hawkridge et al. 2008). The estimated incidence rate of tuberculosis disease in children aged younger than five years is about 2 500/100 000 per annum, which is very high (Hawkridge et al. 2008).

In addition to the trial collecting information on tuberculosis, demographics and socio-economic status, information was collected on previous helminth treatment. This study thus provided an opportunity to test the hypothesis that helminth treatment reduces the risk of tuberculosis disease in young children from a high tuberculosis risk population.

1.1 Rationale

It is important to establish whether children who are treated for helminth infection are protected against tuberculosis.

As tuberculosis is a common disease, a reduction in tuberculosis cases due to introduction of mass deworming programmes in children younger than five years of age could significantly decrease the burden of tuberculosis disease in this age group. This would be strong motivation to introduce regular deworming programmes to include preschool children and include mass deworming treatments in immunisation campaigns.

1.2 Objective

The objective is to determine the association between past helminth treatment and the risk of tuberculosis disease in young children from a high-risk tuberculosis population.

1.3 Study hypothesis

In a high-risk tuberculosis population, young children who have been vaccinated with BCG at birth and have been treated for helminth infection will have lower odds of tuberculosis disease than children who have been vaccinated with BCG at birth but not treated for helminth infection.

2. LITERATURE REVIEW

2.1 Objective of literature review

The objective of this literature review was to assess existing evidence of the effect of chronic helminth infection on the immune system, as well as evidence of a possible association between tuberculosis and helminth infection or helminth treatment, to critically appraise this evidence and to summarise the outcomes of this research.

2.2 Search strategy

Using the key words “helminth infection” or “worm infestation” or “helminth treatment” and “tuberculosis” or "TB", the following resources were searched to identify existing evidence of an association between helminth infection and tuberculosis:

Medline / Pubmed

EMBASE

Cochrane Library

Google

In addition, specialist journals on infectious disease were scanned manually and physicians were asked for guidance by means of personal communication.

2.3 Summary of relevant research

Helminth infections are common in large parts of the world and it is estimated that more than a quarter of the world’s population are infected. This burden is greatest in developing countries especially Africa, South-East Asia and South

America where the burden of other infectious diseases, including tuberculosis, is also high (Borkow et al. 2000; Bundy et al. 2000).

During a period of 15 years, about 60 000 Ethiopians emigrated from Ethiopia to Israel. More than 80% of these immigrants were infected with a least one helminthic parasite.

The immigration of these Ethiopians allowed researchers the opportunity to investigate the effect of chronic immune activation caused by helminth infection on immune responses and the ability of the immune system to cope with infections (Borkow et al. 2000).

Three groups were identified and included in the study. The first group comprised recently immigrated Ethiopians with helminth infection, before deworming. The second group comprised Ethiopians that had been living in Israel for at least three years, had been treated for helminth infection and were at the time of the study free of helminth infection. The third group comprised healthy Israeli-born helminth free individuals. The immune profiles of the three groups were analysed and compared (Borkow et al. 2000).

The study showed a clear dysregulation of several immune parameters in those Ethiopians recently immigrated to Israel compared to the group that had been born in Israel. The differences between the group who had immigrated to Israel at least three years before the study and the group who had been born in Israel were not as great, suggesting that the immune profiles of the Ethiopians gradually returned to normal after immigration to Israel (Borkow et al. 2000).

The researchers concluded that although the improvement in health care and nutrition would have contributed to an improvement in the immune system, eradication of helminth infection was the most important factor in this improvement. (Borkow et al. 2000).

The health benefits of deworming preschool children have been studied in eastern Uganda. A randomised controlled trial was used to investigate the effect of including an anthelmintic in a new health programme for children. The study population was cluster randomised by parish.

Out of the 48 parishes included in the new health programme, 24 parishes offered children aged between one and seven years anthelmintic treatment in addition to standard services on child health days, over a three-year period (Alderman et al. 2006).

The main outcome of the study was weight gain. The results of the study showed about a 10% increase in weight gain in children from the parishes that had been randomised to offer an additional anthelmintic treatment to standard services: [166 g per child per year; (95% CI 16–316)] above expected weight gain when anthelminic treatment was given bi-annually. The weight gain was 5% if the treatment was given annually (Alderman et al. 2006).

The researchers concluded that regular deworming of preschool children, as part of regularly scheduled health services, was practical and resulted in weight gain (Alderman et al. 2006).

The question has been raised in the research community of additional health benefits of deworming young children including possible protection against tuberculosis disease (Bundy et al. 2000; Tristao-Sa et al. 2002; Elias et al. 2006).

Available epidemiological data regarding associations between helminth infection and tuberculosis are largely observational. Two previous studies have shown a significantly higher prevalence of helminth infection in patients with pulmonary tuberculosis compared to control groups as shown in Table 1 (Tristao-Sa et al. 2002; Elias et al. 2006).

Table 1: Summary of previous studies

Author	**Study population**	**Study design**	**Potential confounders** (not adjusted for)	**Effect**
Tristao-Sa (2002)	Adults (hospitalised)	Matched case control	- nutritional status - HIV status	OR 5.19 (2.33 – 11.69)
Elias (2006)	Adults & older children (students)	Matched case control		OR 4.2 (2.7 – 8.7)

Between 1997 and 1999, researchers conducted a case control study in hospitalised patients in Brazil. Stool samples were examined for intestinal nematodes in 57 cases of pulmonary tuberculosis and 86 randomly selected stool examinations from patients with other diseases. The control group individuals were matched to the cases by age, gender and location. The results showed that prevalence of helminths was five times higher in the patients with pulmonary tuberculosis than in the control group: (OR 5.19; 95% CI 2.33 - 11.69) (Tristao-Sa et al. 2002). Although the cases and controls were matched by age, gender and neighbourhood, these results could have been confounded by HIV status which was not evident in the article. As the patients were from the same community it is unlikely that socio-economic status was a confounder.

This result was confirmed in a study done by Elias between October 1999 and 2002 in Ethiopia, where both tuberculosis and helminth infections are endemic (Elias et al. 2006). The study design was case control: 230 cases with clinical features of tuberculosis and smear positive sputum were compared to 510 controls who were selected from the same household as the case, had been resident in the same house for at least 12 months and were found to be free of any signs and symptoms of tuberculosis on normal physical examination.

The researchers found a strong association (OR 4.2; 95% CI 2.7 – 5.9) between tuberculosis and helminth infection and concluded that intestinal

helminth infection together with HIV infection, poverty and poor living conditions may be an important risk factor for tuberculosis (Elias et al. 2006). Age, gender and HIV status were treated as potential confounding variables. The researchers adjusted for their possible effect by including them in the logistic regression model. As the controls were selected from the same household, it is unlikely that confounding by nutritional status would have influenced these results.

These results support the hypothesis that the immune perturbation induced by helminth infection may lessen the host's response to *Mycobacterium tuberculosis*, thus facilitating infection and progression to tuberculosis disease. If this hypothesis holds, eradication of helminth infections could have a significant impact on control of tuberculosis in the developing world (Tristao-Sa et al. 2002; Bentwich et al. 1999).

However, although the studies mentioned above found strong associations between helminth infection and tuberculosis disease in adults and older children (age range 11 to 79 years), with ORs of 5.19 and 4.2 respectively, little is know about the effect of helminth treatment on the incidence of tuberculosis disease in children or adults.

The first trial to examine the effects of helminths and deworming on tuberculosis among young children is currently being conducted in Uganda. The study is a randomised controlled trial which will allow the role of helminths and the benefits of deworming to be determined in relation to infectious diseases including tuberculosis, and the effect on anaemia, growth and intellectual development is to be assessed. The trial has three randomised, double blinded, placebo controlled interventions. Pregnant mothers are randomised to receive either albendazole or placebo and praziquantel or placebo. At the age of 15 months their children are randomised to receive either albendazole or placebo given every three months; to continue until the child is five (Elliot et al. 2007).

In the absence of results from randomised trials, observational data may help to strengthen the case for an additional benefit of deworming.

3. METHODS

This study was conducted in the Boland Overberg region located in the Western Cape Province of South Africa, in the municipal districts of Breede River Winelands, Breede Valley and Witzenberg. The economy is based on agriculture and tourism resulting in the population being small and dispersed. The regional centre is the town of Worcester.

3.1 Study design

A case control study nested within a cohort recruited for a separate randomised control trial to compare two methods of administering BCG vaccination as described earlier was carried out.

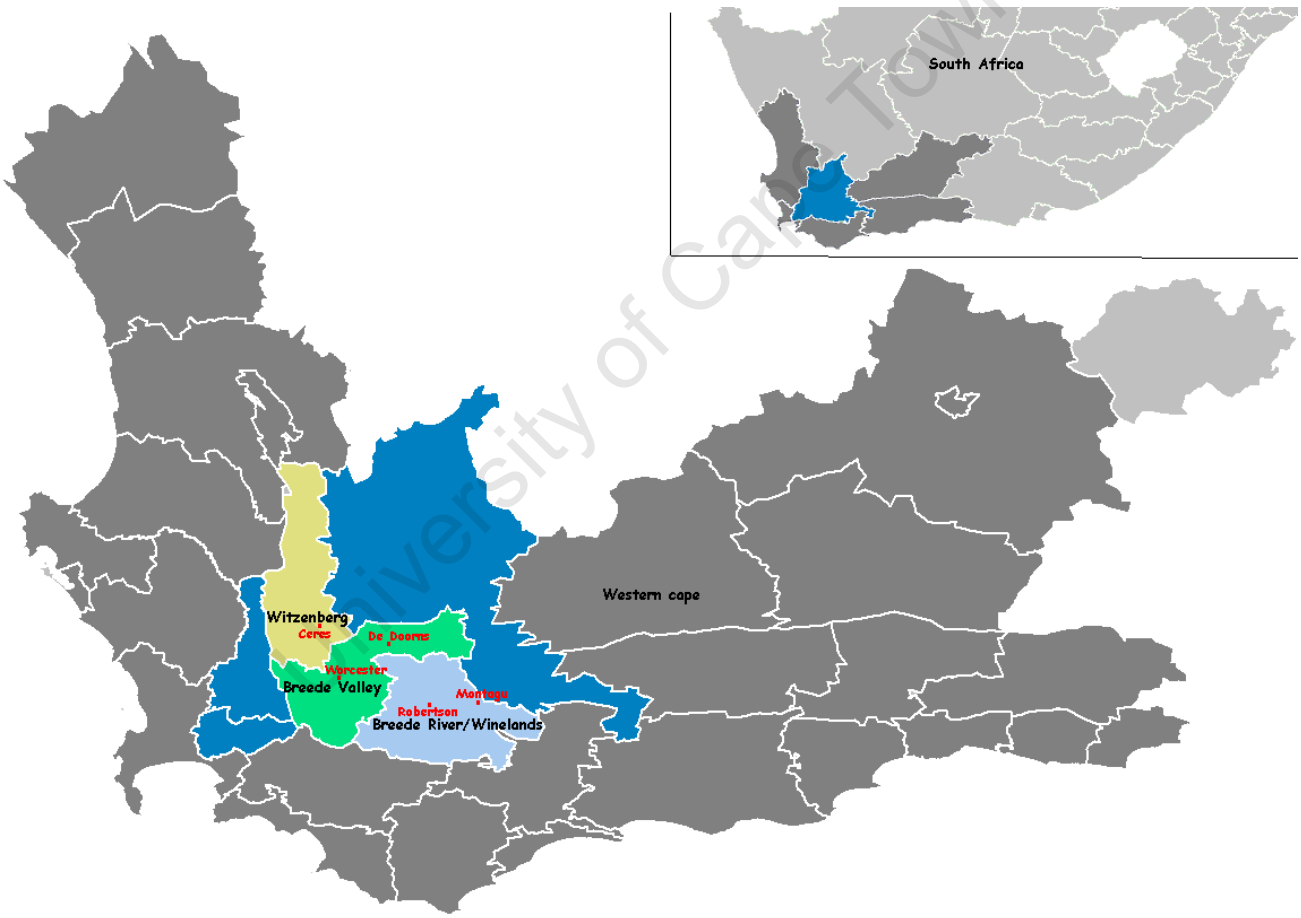
3.2 Study site and study population

The study was carried out in a high tuberculosis burden population. The smear positive detection rate of tuberculosis in this area was 602/100 000 in 2004 (Nicol et al. 2008). The population has a relatively low HIV sero-prevalence for South Africa: between 5.7 and 6.2% of pregnant women attending public health service antenatal clinics (Hawkridge et al. 2008). The estimated incidence rate of tuberculosis disease in children aged less than five years is about 2 500/100 000 per annum, which is very high (Hawkridge et al. 2008).

The comparison of the two methods of administering BCG was needed as the intradermal method of administration of BCG is almost universally accepted over percutaneous administration without direct evidence from a clinical trial. The reasoning behind this was that although it was easier to administer percutaneous BCG, the intradermal method provided for a more accurate dose.

In 1999 the South African Department of Health decided to fall in line with World Health Organization (WHO) recommendations to change the method of BCG vaccination from percutaneous to intradermal. However, it was the view that additional research on the methods of administering BCG vaccination should be carried out. This trial provided the opportunity to carry out the sub-study described in this dissertation.

Figure 1: Map of the Western Cape showing the Boland Overberg region



3.3 Sample size and randomisation of the cohort study

The study cohort comprised 11 680 neonates, vaccinated with BCG within 24 hours of birth, whose mother had signed informed consent and were enrolled in the study. Children excluded were those who were not eligible to receive routine BCG vaccination within 24 hours of birth, were not from the study area or were not born at one of five public maternity health units.

The randomisation sequence was generated by the study statistician in Microsoft Excel. The randomisation was by week and balanced in eight-week blocks of four intradermal and four percutaneous in one eight-week cycle.

The children were enrolled by midwives and study counsellors who were aware of the week's vaccine allocation. Allocation concealment and masking did not take place.

3.4 Surveillance for tuberculosis and referral to the case verification ward

The cohort was followed up for at least two years after BCG vaccination. Active surveillance for tuberculosis disease was used for the first three months. A total of 4 851 children were screened for incident illness that could have been tuberculosis when they attended a local health facility for routine vaccination visits at six, 10 and 14 weeks of age. Any children with a history of exposure to tuberculosis or those who presented with symptoms suggestive of tuberculosis were admitted to a specialised tuberculosis case verification ward at Brewelskloof Hospital, Worcester. Passive surveillance was based on scanning hospital admission lists, tuberculosis registers and mortality records for illness suggestive of tuberculosis or death. All surveillance events that were reported that could have been tuberculosis were investigated by a member of the study staff.

Table 16: Potential confounders

(primary analysis association between helminth Rx and the co-variables)

Co-variable	Helminth Rx (n=152)	No helminth Rx (n=283)	OR	95% CI
Age at TB investigation (median) (mths)	22.55	15.37	1.15	1.11 – 1.19
Gender (% male)	53.29	49.82	1.15	0.77 – 1.71
Ethnicity (% coloured)	82.24	81.98	1.02	0.61 – 1.70
Birth weight (mean) (kg)	2.83	2.80	1.12	0.77 – 1.64
Low birth weight (% birth weight < 2.5 kg)	23.68	24.73	0.94	0.60 – 1.50
Mother's age at child's birth (mean)	25.56	25.53	1.00	0.97 – 1.03
Gestation (median) (weeks)	39	39	1.05	0.97 – 1.15
Early birth (% born before 37 weeks)	20.39	21.55	0.93	0.57 – 1.52
Type of housing (% living in an informal dwelling)	7.24	9.89	0.71	0.34 – 1.47
Type of community (% living on a farm)	21.71	25.09	0.84	0.52 – 1.32
Number of occupants (median)	5	6	0.97	0.90 – 1.04
haz (mean)	-1.17	-1.14	0.99	0.86 – 1.13
Birth site: (% born in Worcester)	44.74	57.96	1.00	
Birth site: (% born in Ceres)	17.76	18.02	1.28	0.74 – 2.20
Birth site: (% born in Robertson)	25.00	13.78	2.35	1.39 – 3.99
Birth site: (% born Montagu)	12.50	10.24	1.58	0.83 – 3.01
HIV status (% HIV exposed)	2.63	2.47	1.07	0.31 – 3.07

IQR – interquartile range

CI – confidence interval

4.4.2 Univariate analysis

Table 17: Association between helminth treatment and tuberculosis
Model 1 (primary analysis n=510)

Variable	OR	95% CI
Helminth treatment	1.04	0.71 – 1.51

The univariate unadjusted analysis shows no association between tuberculosis and helminth treatment as shown in Table 17.

4.4.3 Multivariate analysis

The logistic regression analysis was performed on 435 children who had a full set of data for all variables included in this analysis.
Observations that had missing data were excluded in the logistic regression analyses as shown in Tables 11 and 12.

Table 18: Association between helminth treatment and tuberculosis
Model 2 (primary analysis n=435)

Variable	OR	95% CI	Akaike's information criterion (AIC)
Helminth treatment	1.05	0.70 – 1.59	570.55

Model building proceeded by adding other risk factors to the model with helminth treatment, including the variables that proved to have a significant univariate association with tuberculosis disease as identified in Table 13.

The model was increased by adding each of the co-variables separately. The best model was selected using the lowest Akaike's information criterion (AIC) and the likelihood ratio test p value was used to determine if this was a statistically significant improvement.

Table 19: Primary analysis

Model 3 (adding each variable to the model of helminth Rx)

Variable	lrtest p-value	AIC
Age at investigation	0.11	569.94
Gender	0.02	566.69
Ethnicity	0.28	571.36
Birth weight	0.06	569.13
Low birth weight	0.17	570.69
Mother's age	0.78	572.47
Gestation	0.18	570.71
Early birth	0.09	569.70
Housing	0.71	572.41
Type of community	0.33	571.61
Number of occupants	0.03	567.62
haz	<0.001	559.28
HIV status	0.55	572.19
Birth site	0.05	568.59

Adding haz results in the most significant improvement to the model that only contained helminth treatment: AIC 559.28; lrtest p value < 0.001 as shown in Table 19.

Table 20: Primary analysis adjusted for haz

Model 4 (adding each variable to the model of helminth Rx and haz)

Variable	lrtest p-value	AIC
Age at investigation	0.23	559.86
Gender	0.02	556.18
Ethnicity	0.43	560.66
Birth weight	0.85	561.24
Low birth weight	0.88	561.26
Mother's age	0.56	560.94
Gestation	0.41	560.61
Early birth	0.23	559.76
Housing	0.82	561.23
Type of community	0.45	560.72
Number of occupants	0.02	555.79
HIV status	0.77	561.19
Birth site	0.11	559.29

Adding number of occupants results in the most significant improvement to the model that contained helminth treatment and haz: AIC 555.79; lrtest p value = 0.02 as shown in Table 20.

Table 21: Primary analysis adjusted for haz and number of occupants

Model 5 (adding each variable to the model of helminth Rx, haz and number of occupants)

Variable	lrtest p-value	AIC
Age at investigation	0.23	556.31
Gender	0.02	552.55
Ethnicity	0.61	557.54
Birth weight	0.96	557.79
Low birth weight	0.97	557.97
Mother's age	0.61	557.54
Gestation	0.50	557.35
Early birth	0.27	556.60
Housing	0.01	557.78
Type of community	0.92	556.88
HIV status	0.70	557.64
Birth site	0.15	556.42

Adding gender results in the most significant improvement to the model that contains helminth treatment, haz and number of occupants: AIC 552.55; lrtest p value = 0.02 as shown in Table 21.

Table 22: Primary analysis adjusted for haz, number of occupants and gender

Model 6 (adding each variable to the model of helminth treatment, haz, number of occupants and gender)

Variable	lrtest p-value	AIC
Age at investigation	0.23	553.09
Ethnicity	0.67	554.37
Birth weight	0.73	554.44
Low birth weight	0.86	554.52
Mother's age	0.50	554.10
Gestation	0.45	553.98
Early birth	0.29	553.42
Housing	0.99	554.55
Type of community	0.38	553.78
HIV status	0.66	554.35
Birth site	0.10	552.36

Adding birth site is an improvement on the model that contains helminth treatment, haz and number of occupants and gender: AIC 552.36; lrtest p value = 0.10 as shown in Table 22. Although this is not a statistically significant result at the 95% level of confidence it was felt that it should be included in the final model as it had a positive association with tuberculosis in the baseline univariate analysis as shown in Table 13.

There is no further improvement to the model by adding additional variables. The best model to examine a relationship between tuberculosis and helminth treatment is a model that includes helminth treatment, haz, number of occupants living in the same dwelling as the child, gender and birth site as shown in Table 23.

Table 23: Primary analysis final model adjusted for haz, number of occupants, gender and birth site

Model 7 (AIC 552.36)

Variable	OR	95% CI
haz	0.78	0.67 – 0.91
Number of occupants	1.10	1.01 – 1.19
Gender	1.67	1.11 – 2.51
Birth site Worcester	1.0	
Birth site Ceres	1.56	0.88 – 2.77
Birth site Robertson	1.94	1.07 – 3.51
Birth site Montagu	1.38	0.71 – 2.71
Helminth treatment	**0.98**	**0.64 – 1.51**

The validity of the model was examined using a plot of residuals versus the linear component of the model as shown in Appendix A figure 3. Pearson chi-square p value of 0.3331 indicates a reasonably good fit, $p > 0.05$.

After adjusting for the effect of haz, number of occupants living in the same dwelling as the child, gender and birth site, there was still no evidence of an association between helminth treatment and tuberculosis disease.

Although the OR changed from an unadjusted OR of 1.05 to 0.98 this is only a difference of 6.6% and the OR is very close to 1 with a 95% CI that includes 1, indicating that there is no relationship between tuberculosis and helminth treatment.

The observed relative difference of 5% is much smaller than the hypothesized 50% relative effect on which the initial power calculations were done at the design phase of the study. Given the proportion of cases and controls treated

for helminth infection in this analysis of 328 cases of tuberculosis and 182 controls of not TB, the study is only 3% powered to prove this reduced relative difference statistically significant at the 5% level, as shown in Tables 24 and 25.

Table 24: Power of the study
(primary univariate analysis n=510)

Proportion of cases treated for helminth infection	Proportion of controls treated for helminth infection	OR	95% CI	Power
35.98%	35.16%	1.05	0.70 – 1.59	3%

Table 25: Power of the study
(primary multivariate analysis n=435)

Proportion of cases treated for helminth infection	Proportion of controls treated for helminth infection	OR	95% CI	Power
35.36%	34.19%	1.05	0.70 – 1.60	3%

This study showed a much smaller difference in proportion of cases and controls exposed to helminth treatment than used in the original sample size calculations. Thus, the study ended up being under-powered. Table 24 shows that for the univariate analysis of the association between helminth treatment and tuberculosis, there was only 3% power to prove the observed effect as measured by an OR of 1.05 to be statistically significant at the 5% level. This power remains at 3% for the reduced sample used in the multivariate model as shown in Table 25.

Once the best model had been selected using 435 children who had a full set of data for all variables included in this sub-set, as shown in Table 23, the multivariate analysis was repeated using as many observations as possible as shown in Table 26.

Table 26: Primary analysis adjusted for haz, number of occupants, gender and birth site (n=493)

Model 8 (AIC 552.36)

Variable	OR	95% CI
haz	0.79	0.69 – 0.91
Number of occupants	1.11	1.02 – 1.19
Gender	1.56	1.07 – 2.29
Birth site Worcester	1.0	
Birth site Ceres	1.38	0.81 – 2.34
Birth site Robertson	1.68	0.97 – 2.92
Birth site Montagu	1.42	0.77 – 2.64
Helminth treatment	**1.03**	**0.69 – 1.53**

The result is similar to the model that analysed 435 children. There is no relationship between helminth treatment and tuberculosis. However birth site is no longer significant at the 95% level of confidence.

The validity of the model was examined using a plot of residuals versus the linear component of the model as shown in Appendix A figure 4. Pearson chi-square p value of 0.3786 indicates a reasonably good fit, $p > 0.05$.

4.5 Secondary analysis

(Including those classified as “unlikely TB” with the control group).

Baseline characteristics of the secondary analysis are similar to the baseline characteristics of the children included in the primary analysis as shown in Table 26. Both univariate analyses show that tuberculosis is associated with haz, gender and number of occupants sharing the same dwelling as the child. However birth site is not statistically significantly associated with tuberculosis in the secondary analysis as it was in the primary analysis and HIV status now has a statistically significant association with tuberculosis.

Table 27: Baseline characteristics in cases and controls

(secondary analysis n=847)

(Univariate logistic regression of an association between co-variable and tuberculosis n= 847)

Variable	Total (n=847)	Cases (n=328)	Controls (n=519)	OR	95% CI
Age at TB investigation (median & range) (mths)	18.37 (9–47.53)	18.02 (9.17-47.53)	18.9 (9.0-47.50)	0.99	0.97 – 1.01
Gender (% male)	50.89	54.88	45.12	1.2	0.98 – 1.71
Ethnicity (% coloured)	84.03	83.91	84.11	0.98	0.67 – 1.45
Birth weight (mean & range) (kg)	2.79 (1.08–5.55)	2.75 (1.08-5.55)	2.82 (1.26-4.44)	0.79	0.61 – 1.03
Low birth weight (% birth weight < 2.5 kg)	27.27	28.66	26.4	1.12	0.82 – 1.53
Mother's age (mean & range)	25.85 (14.18–46.56)	25.65 (15.04-42.17)	25.98 (14.18-46.56)	0.99	0.97 – 1.01
Gestation (median & range) (weeks)	39 (29–44)	39 (30-44)	39 (29-44)	0.99	0.94 – 1.05
Early birth (% born before 37 weeks)	21.96	23.44	21.00	1.15	0.83 – 1.16
Type of housing (% living in an informal dwelling)	10.39	9.45	10.98	0.85	0.53 – 1.34
Type of community (% living on a farm)	24.32	23.78	24.66	0.95	0.69 – 1.32
Number of occupants (median & range)	5 (1–20)	6 (1-20)	5 (1-14)	1.05	1.00 – 1.12
HAZ (mean & range)	-1.08 (-5.05–5.07)	-1.45 (-5.05-2.34)	-0.94 (-4.95-5.07)	0.80	0.72 – 0.88
Birth site: (% born Worcester)	51.59	48.48	53.56	0.82	0.62 – 1.08
Birth site: (% born Ceres)	19.60	20.12	19.27	1.06	0.75 – 1.49
Birth site: (% born Robertson)	17.24	19.21	15.99	1.25	0.87 – 1.79
Birth site: (% born Montagu)	11.57	12.20	11.18	1.10	0.72 – 1.70
Helminth treatment (% treated)	38.61	35.98	40.27	0.83	0.63 – 1.11
HIV status (% infected)	2.13	3.58	1.22	3.01	1.10 – 8.22

IQR – interquartile range

CI – confidence interval

All co-variables were tested for potential confounding using the method described earlier. Number of occupants sharing the same dwelling as the child and haz are significantly related with tuberculosis in those not exposed to helminth treatment as shown in Table 28. Age and birth site are significantly related to exposure to helminth treatment as shown in Table 29. None of the variables satisfies both criteria for being a potential confounder.

Table 28: Potential confounders
(secondary analysis not exposed to helminth Rx)

Variable	Cases (n=210)	Controls (n=310)	OR	95% CI
Age at TB investigation (median) (mths)	15.72	14.78	1.01	0.98 – 1.04
Gender (% male)	51.90	46.77	1.23	0.87 – 1.74
Ethnicity (% coloured)	84.23	83.45	1.06	0.65 – 1.73
Birth weight (mean) (kg)	2.75	2.81	0.80	0.57 – 1.12
Low birth weight (% birth weight < 2.5 kg)	28.10	27.10	1.05	0.71 – 1.56
Mother's age (mean)	25.68	25.64	1.00	0.97 – 1.03
Gestation (median) (weeks)	39	38	0.99	0.92 – 1.06
Early birth (% born before 37 weeks)	24.29	20.97	1.21	0.80 – 1.84
Type of housing (% living in an informal dwelling)	9.52	11.29	0.83	0.46 – 1.48
Type of community (% living on a farm)	25.71	26.45	0.96	0.65 – 1.43
Number of occupants (median)	6	5	1.08	1.01 – 1.16
haz (mean)	-1.30	-0.93	0.84	0.74 – 0.95
Birth site: (% born Worcester)	55.72	55.51	1.00	
Birth site: (% born Ceres)	20.95	22.26	0.94	0.60 – 1.46
Birth site: (% born Robertson)	13.81	11.29	1.22	0.71 – 2.10
Birth site: (% born Montagu)	9.52	10.97	0.86	0.47 – 1.58
HIV status (% infected)	3.00	0.67	4.56	0.91 – 22.83
haz (mean)	-1.30	-0.93	0.84	0.74 – 0.95

IQR – interquartile range

CI – confidence interval

Table 29: Potential confounders

(secondary analysis association between helminth Rx and the co-variables)

Variable	Helminth Rx (n=327)	No helminth Rx (n=520)	OR	95% CI
Age at TB investigation (median) (months)	22.90	15.36	1.15	1.12 – 1.18
Gender (% male)	54.13	48.85	1.24	0.94 – 1.63
Ethnicity (% coloured)	88.44	83.77	1.05	0.71 – 1.55
Birth weight (mean) (kg)	2.81	2.78	1.08	0.83 – 1.41
Low birth weight (% birth weight < 2.5 kg)	26.91	27.50	0.97	0.71 – 1.32
Mother's age (mean)	26.16	25.66	1.01	0.99 – 1.03
Gestation (median) (weeks)	39	38	1.05	0.99 – 1.11
Early birth (% born before 37 weeks)	21.41	22.31	0.95	0.68 – 1.33
Type of housing (% living in an informal dwelling)	10.09	10.58	0.95	0.60 – 1.50
Type of community (% living on a farm)	21.41	26.15	0.78	0.55 – 1.07
Number of people living in the same dwelling (median)	6	5	0.98	0.92 – 1.03
haz (mean)	-1.09	-1.07	0.99	0.90 – 1.10
Birth site: (% born Worcester)	45.25	55.58	1.00	
Birth site: (% born Ceres)	16.21	21.73	0.92	0.63 – 1.34
Birth site: (% born Robertson)	25.08	12.31	2.50	1.71 – 3.67
Birth site: (% born Montagu)	13.46	10.38	1.59	1.02 – 2.48
HIV status (% infected)	2.98	1.61	1.88	0.72 – 4.92

IQR – interquartile range

CI – confidence interval

4.5.1 Univariate analysis

Table 30: Association between helminth treatment and tuberculosis
Model 9 (secondary analysis n=847)

Variable	OR	95% CI
Helminth treatment	0.83	0.63 – 1.11

The univariate unadjusted analysis shows a 17% relative reduction in the odds of tuberculosis. However, this result is not statistically significant at the 5% level of significance as shown in Table 30.

4.5.2 Multivariate analysis

The logistic regression analysis was performed on 724 children who had a full set of data for all variables included in this sub-set.

Table 31: Association between helminth treatment and tuberculosis
Model 10 (secondary analysis n=724)

Variable	OR	95% CI	AIC
Helminth treatment	0.83	0.61 – 1.14	968.86

Model building proceeded by adding other risk factors to the model with helminth treatment, including variables that proved to have a significant univariate association with tuberculosis disease, including those identified in Table 27.

The model was increased by adding each of the co-variables separately. The best model was selected by using the lowest Akaike's information criterion (AIC) and the lrtest p value was used to determine if this was a statistically significant improvement.

Table 32: secondary analysis

Model 11 (adding each variable to the model of helminth Rx)

Variable	lrtest p-value	AIC
Age at investigation	0.08	967.81
Gender	0.05	967.07
Ethnicity	0.77	970.78
Birth weight	0.16	968.92
Low birth weight	0.67	970.68
Mother's age	0.45	970.28
Gestation	0.50	970.41
Early birth	0.12	968.40
Housing	0.23	969.45
Type of community	0.22	970.64
Number of occupants	0.03	966.32
haz	<0.001	952.85
HIV status	0.15	968.75
Birth site	0.20	970.23

Adding haz results in the most significant improvement to the model that only contained helminth treatment: AIC 952.85; lrtest p value < 0.001 as shown in Table 32.

Table 33: Secondary analysis adjusted for haz

Model 12 (adding each variable to the model of helminth Rx and haz)

Variable	lrtest p-value	AIC
Age at investigation	0.17	952.97
Gender	0.07	951.46
Ethnicity	0.45	954.27
Birth weight	0.51	954.41
Low birth weight	0.23	953.41
Mother's age	0.18	953.05
Gestation	0.93	954.84
Early birth	0.95	953.89
Housing	0.19	953.11
Type of community	0.94	954.84
Number of occupants	0.02	949.27
HIV status	0.26	953.57
Birth site	0.28	955.00

Adding number of occupants results in the most significant improvement to the model that contained helminth treatment and haz: AIC 949.27; lrtest p value = 0.02 as shown in Table 33.

Table 34: Secondary analysis adjusted for haz and number of occupants

Model 13 (adding each variable to the model of helminth Rx, haz and number of occupants)

Variable	lrtest p-value	AIC
Age at investigation	0.17	949.37
Gender	0.07	947.94
Ethnicity	0.99	950.27
Birth weight	0.47	950.74
Low birth weight	0.21	949.71
Mother's age	0.23	949.80
Gestation	0.85	951.23
Early birth	0.40	950.56
Housing	0.33	950.30
Type of community	0.75	951.16
HIV status	0.21	949.69
Birth site	0.33	951.85

Adding gender results in the most significant improvement to the model that contains helminth treatment, haz and number of occupants: AIC 947.94; lrtest p value = 0.07 as shown in Table 34.

Table 35: Primary analysis adjusted for haz, number of occupants and gender

Model 15 (adding each variable to the model of helminth treatment, haz, number of occupants and gender)

Variable	lrtest p-value	AIC
Age at investigation	0.19	948.26
Ethnicity	0.31	948.91
Birth weight	0.65	949.74
Low birth weight	0.30	948.86
Mother's age	0.16	947.99
Gestation	0.89	949.92
Early birth	0.40	949.23
Housing	0.29	948.83
Type of community	0.82	949.89
HIV status	0.22	948.42
Birth site	0.33	950.53

There is no further improvement to the model by adding additional variables. The best model to examine a relationship between tuberculosis and helminth treatment is a model that includes helminth treatment, haz, number of occupants living in the same dwelling as the child and gender as shown in Table 36.

Table 36: Secondary analysis adjusted for haz, number of occupants and gender

Model 16 (AIC 947.94)

Variable	OR	95% CI
haz	0.79	0.71 – 0.88
Number of occupants	1.08	1.01 – 1.44
Gender	1.33	0.98 – 1.81
Helminth treatment	**0.82**	**0.60 – 1.13**

After adjusting for the effect of haz, gender (not significant at the 5% level of significance) and number of occupants living in the same dwelling as the child, the relationship between helminth treatment and tuberculosis disease remains unchanged as shown in Table 36.

The validity of the model was examined using a plot of residuals versus the linear component of the model as shown in Appendix A figure 5. Pearson chi-square p value of 0.4347 indicates a reasonably good fit, $P > 0.05$.

Given the proportion of cases and controls treated for helminth infection in this analysis of 328 cases of tuberculosis and 519 controls of unlikely or not TB, the study is only 22% powered to prove this reduced relative difference significant at the 5% level of significance, in the unadjusted univariate analysis. This power reduces to 19% in the adjusted multivariate analysis as shown in Tables 37 and 38.

Table 37: Power of the study
(secondary analysis n=847)

Proportion of cases treated for helminth infection	Proportion of controls treated for helminth infection	OR	95% CI	Power
35.98	40.27	0.83	0.63 – 1.11	22%

Table 38: Power of the study
(secondary analysis n=724)

Proportion of cases treated for helminth infection	Proportion of controls treated for helminth infection	OR	95% CI	Power
35.36	39.64	0.82	0.60 – 1.13	19%

Once the best model had been selected using the 724 children who had a full set of data for all variables included in this sub-set as shown in Table 36, the multivariate analysis was repeated using as many observations as possible as shown in Table 39.

Table 39: Secondary analysis adjusted for haz, number of occupants and gender (n=822)

Model 17

Variable	OR	95% CI
haz	0.79	0.72 – 0.88
Number of occupants	1.07	1.01 – 1.13
Gender	1.30	0.97 – 1.72
Helminth treatment	**0.85**	**0.63 – 1.14**

The result is similar to the model that analysed 724 children. There is no relationship between helminth treatment and tuberculosis, the relative reduction in tuberculosis odds is 15% but this was not a statistically significant result as shown in Table 39.

5. DISCUSSION

The principal findings of the primary analysis of this study do not demonstrate that children who have been treated for helminth infection and live in a community with a high tuberculosis burden are better protected against tuberculosis disease than those not treated for helminth infection. While the secondary analysis which included those classified as unlikely tuberculosis in the control group showed a decrease in tuberculosis odds of 17% in the univariate analysis and 15% in the adjusted multivariate analysis, this result was not statistically significant at the 95% level of significance.

One of the strengths of this study was that all the children included in the analysis were investigated for tuberculosis in a hospital ward that had been specifically equipped to perform tests used in the diagnosis of tuberculosis. The procedures and tests were performed by study staff who had been trained in protocol and standardised methods. This standardised method of performing the necessary tests would have minimised the diagnostic measurement error.

As this is a case control study, careful attention was paid to the selection of the control groups. In the primary analysis the control group comprised children who were completely asymptomatic. These children were selected for investigation of tuberculosis as they had been exposed to an adult case of tuberculosis. It was felt that although they could have been less healthy than, and therefore not fully representative of, the underlying population of this age group, it was unlikely that this would have changed the results of the study as the OR was very close to 1.

The secondary analysis was performed to establish if the result of the primary analysis held true when those classified as “unlikely TB” were included in the control group, as well as to increase the power of the study.

As both helminth infection and tuberculosis are strongly associated with loss of weight and malnutrition, multivariate analysis which adjusted for these variables was used.

Factors that could have influenced these results include temporality; it was not known when the child was treated for helminth infection. This could have been some months before the tuberculosis investigation and thus the child had the opportunity to become re-infected with helminths. In addition, this study was not equipped to measure helminth infection prevalence directly; the exposure variable that was analysed was only of a history of helminth treatment.

One of the difficulties in trying to establish an association between tuberculosis and helminth infection is the fact that they are both chronic diseases, therefore it cannot be established with certainty whether helminth infection preceded tuberculosis or if chronic tuberculosis might have led to an increased risk of helminth infection. It is possible that the tuberculosis disease process might have started before helminth treatment and might have been undetected.

It is also not known if the helminth treatment had been effective. It is possible that there were children who had been given helminth treatment but were still infected with helminths and thus remained at greater risk of tuberculosis than others effectively treated.

To validate the accuracy of the mother's recall of previous treatment for helminth infection, a 10% sample of CRFs were compared to the child's RTHC where a record of helminth treatment was recorded by staff at the local health facility. No discrepancies in the 10% sample check was found.

It is unlikely that the mother of the child would have accessed helminth treatment from a source other than the local health centre, but it is possible and if she had she might not have been able to recall this previous helminth treatment accurately.

The results of our study differed from other reported research in that two previous case control studies (Tristao-Sa et al. 2002; Elias et al. 2006) found strong associations between helminth infection (as established by examination of stools) and tuberculosis. However, the primary objectives of these studies were to study the prevalence of helminth infection in relation to tuberculosis disease. In neither of these two studies is it known if any participants had previously been treated for helminth infection. It is possible that a larger proportion of the controls had been treated for helminth infection and this could have been a factor that protected them from tuberculosis.

The populations that were previously studied were also different from the population that was investigated in this study. Both previous case control studies were conducted in adults and older children with an age range of 10–80 years. In contrast, the primary objective of this case control study was to determine if children who had been treated for helminth infection had a reduced risk of tuberculosis in the first five years of life. The focus of this research was thus not on the prevalence of helminths but rather the effect of helminth treatment on tuberculosis risk in young children.

In addition to the above findings, this study found an association between tuberculosis and malnutrition, gender and birth site.

A haz below -2 describes stunting which implies long term malnutrition and poor health (WHO, 2007). A total of 27.25% of the study population presented with a haz less than -2, indicating a high proportion of chronically malnourished children. There was a large and statistically significant difference in the proportion of children with a haz <-2 between the cases and controls, 33.21% and 13.55% respectively (OR 3.17; 95% CI 1.88 – 5.35). Children who present with a haz of <-2 are three times more likely to develop tuberculosis than children who present with a haz of >-2 indicating a strong relationship between tuberculosis and malnutrition.

The association between malnutrition and tuberculosis has been explored by many researchers and these results support previous findings (Elias et al. 2001; Campbell et al. 2006; Alderman et al. 2006).

This analysis also showed that children born at the Ceres Hospital or Robertson Hospital were more likely to be diagnosed with tuberculosis than those born in Worcester at either the Eden Donges Hospital or Maria Pieterse Maternity Health Unit. The reason for this difference is not known.

This study found a statistically significant difference between the odds of tuberculosis in males and females. Male children were 1.5 times more likely to be diagnosed with tuberculosis than female children. Differences in incidence of tuberculosis by gender have been noted in previous adult and adolescent research, with results indicating that males are more at risk of tuberculosis than females (Holmes et al. 1998; Nissapatorn et al. 2006). However this difference has not been noted in children. In a review of gender differences in the epidemiology of tuberculosis, Holmes et al. (1998) found that differences in incidence between genders became apparent only after adolescence. They concluded that this gender differential after adolescence was probably due to under reporting of female cases of tuberculosis in developing regions.

Data reported from the National Tuberculosis Centre in Malaysia from January, 2001, to December, 2002, found that 68% of the tuberculosis cases reported were male. This report suggested that these differences in gender were probably due to increasing patterns of non-adherence to tuberculosis treatment in males and poor socio-economic conditions (Nissapatom et al. 2006).

These gender differences in adults with tuberculosis are confirmed in a report by the WHO (2002); However, they state that in most settings the higher incidence of tuberculosis in males only begins to appear between the ages of 10 and 16 years.

6. CONCLUSION

The benefits of deworming young children have been well documented: reducing the number of young children infected with helminths will result in improved physical growth and cognition as well as in improved development of children (Alderman et al. 2006).

The South African Department of Health policy and guidelines for regular treatment of school-going children for soil transmitted helminth infection state that regular deworming of children aged two to five years is included in the Integrated Management of Childhood Illness (IMCI) case management guidelines and that every child between the ages of one and five years should receive regular six-monthly deworming.

Although this study did not show any effect of helminth treatment on the risk of tuberculosis in young children, the benefit of deworming on weight gain is sufficient justification for regular deworming.

The primary analysis of this observational study does not support the hypothesis that helminth treatment reduces the risk of tuberculosis disease in young children in a high-risk tuberculosis population. None of the variables met a priori for potential confounding. The result was thus unchanged when adjusted for the effect of haz, number of occupants sharing the same dwelling as the child, gender and birth site.

Although the secondary analysis showed a 17% reduction in tuberculosis odds, this was not a statistically significant result. The result was also essentially unchanged when adjusted for the effect of haz, number of occupants sharing the same dwelling as the child and gender.

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APPENDIX A: MODEL CHECKING GRAPHS

Figure 3: Plot of residuals versus the linear component of the model
(Primary analysis of 435 children)

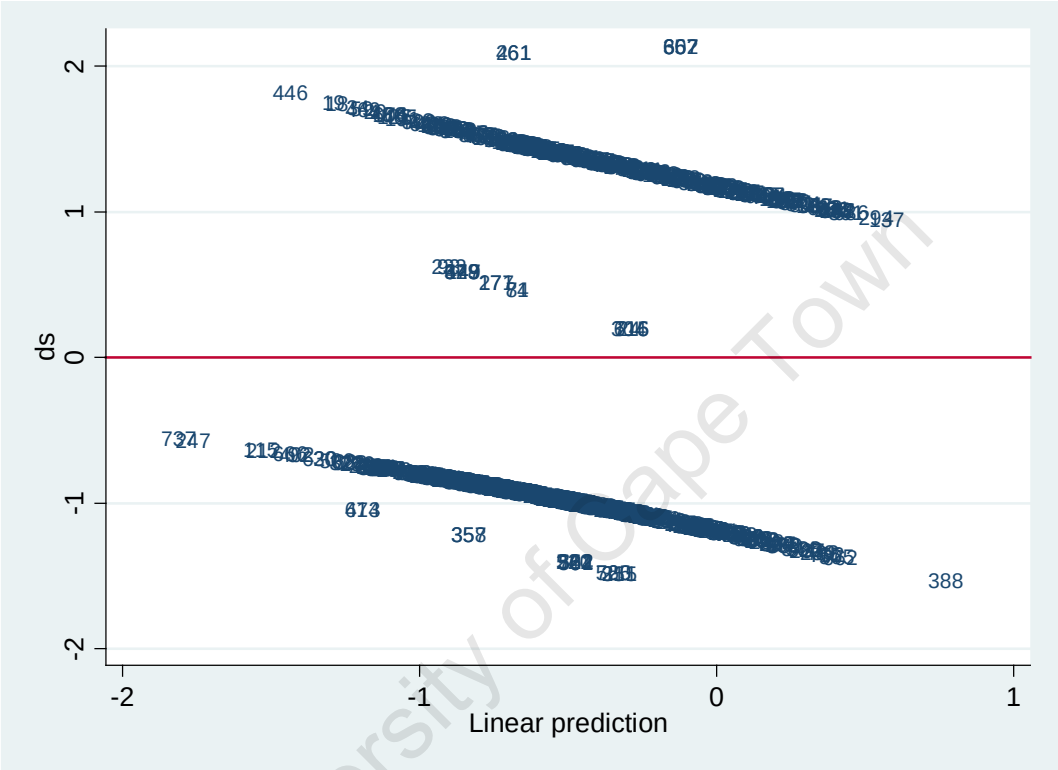

Figure 4: Plot of residuals versus the linear component of the model.
(Primary analysis of 493 children)

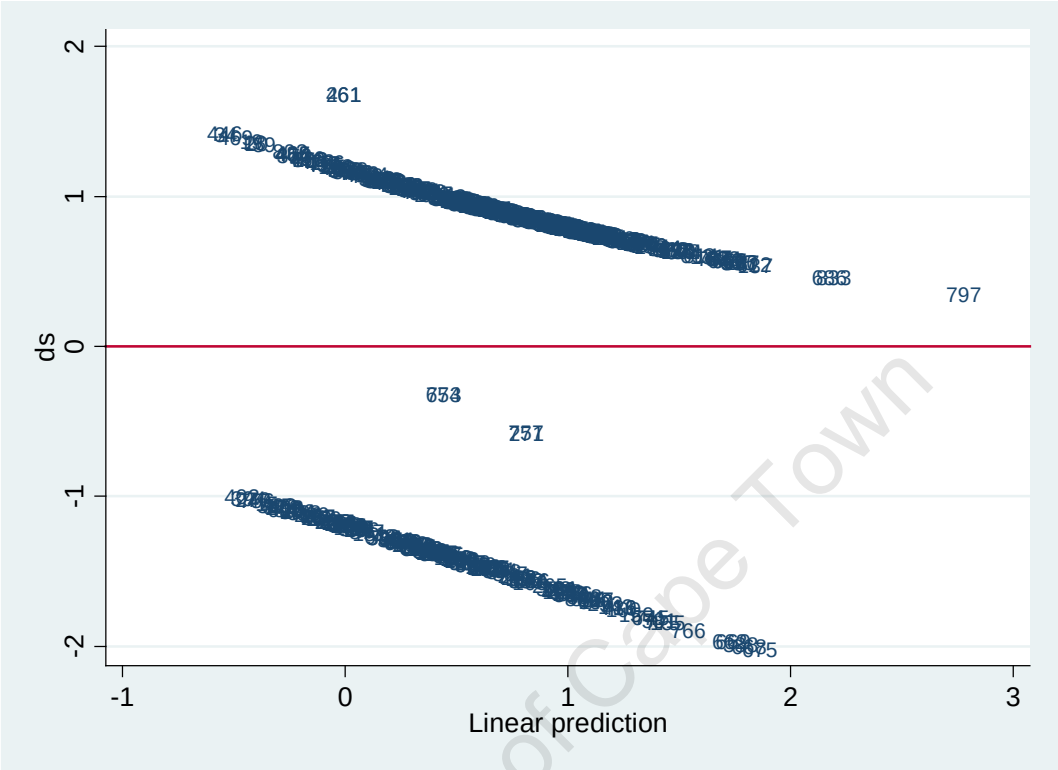

Figure 5: Plot of residuals versus the linear component of the model.
(Secondary analysis of 724 children)