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Cynthia N. Jones Date

Leprosy in the Wake of Helminth Immunomodulation:

A study on the impact of deworming on leprosy outcomes in Vale do Rio Doce, Brazil

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2015

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An abstract of

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Abstract

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By Cynthia N. Jones

Background: Helminthic immunomodulation is believed to affect the human immune response to several diseases of public health importance, including leprosy. The implementation of a four-year school-based deworming and leprosy detection campaign throughout Brazil brings about an important opportunity to study the as-yet unidentified effects of soil-transmitted helminth treatment on the incidence of leprosy and its subtypes.
Methods: We conducted two analyses on incident cases of leprosy retrieved from SINAN, Brazil's nationally notifiable disease surveillance system, between 2002 and early 2017. The first was a difference-in-differences analysis in which trends in leprosy outcomes within Vale Do Rio Doce, Minas Gerais, Brazil, were compared in the years leading up to the commencement of Brazil's national school-based deworming campaign through the period during which anthelmintic treatment actively occurred in selected municipalities. Linear regression was used to compare the effect of school-based deworming on leprosy new case detection rate and percent multibacillary cases in the general population and among children. A spatial hotspot analysis was conducted and evaluated to complement the difference-in-differences models and to determine the effect of school-based deworming on inclusion in a leprosy hotspot.
Results: No significant effect of school-based anthelmintic treatment on new case detection rate, percent of multibacillary cases, or pediatric new cases and percent of multibacillary cases was observed from the aspatial difference-in-differences model. Minor changes in hotspot locations were observed throughout the study period; however, no statistically significant difference in hotspot changes between intervention and control groups was detected.
Conclusions: While no significant effect of anthelmintic treatment on leprosy incidence or percent of multibacillary cases was observed, the cluster analysis did reveal areas of consistently high leprosy transmission. These areas coincide largely with municipalities selected to take part in annual school-based deworming, presenting future opportunities for further integration of programs to eliminate and cooperatively control these diseases. Additional analyses are essential to identify the true effect of anthelmintic treatment on leprosy incidence and polarization, as well as further characterize the relationship between these diseases.

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I would like to thank Dr. Jessica Fairley for giving me guidance and offering her expertise throughout the course of my study. I would also like to thank Dr. Kenneth Castro for his support of my project. Special thanks to our colleagues at the Secretary of Health in Minas Gerais, in particular Dr. Maria Aparecida de Faria Grossi, who allowed us to use the data for this analysis. Lastly, I'd like to thank Dr. Julie Clennon and Dr. Gonzalo Vazquez-Prokopek for their suggestions and guidance on the GIS aspects of the project.

Table of Contents

Chapter I: Literature Review

Leprosy: past and present

Leprosy, also called Hansen's disease, is one of the oldest known diseases in human history (1). Though often considered a disease of the past, transmission of leprosy persists globally, at much lower levels than previously. The disease causes physical disability and prominent skin abnormalities, which often leads to stigmatization and isolation, affecting not only the physical health of the individual, but also their social, mental, economic, and psychologic well-being (1). In 1991, the World Health Assembly passed a resolution to eliminate leprosy as a public health problem by the year 2000, defined as a reduction in leprosy incidence to less than 1 case per 10,000 population (2). Changes accompanying this resolution included widespread access to multi-drug therapy for cases, special action projects for elimination, and leprosy elimination campaigns (2). The next decade saw a rapid decline in global leprosy prevalence, and on a global scale the target was reached by 2000 (3). However, nearly two decades after the set endpoint, a few endemic countries have yet to individually achieve elimination of leprosy as a public health problem. For Brazil, the number of reported new leprosy cases annually has stabilized in recent years just above 25,000 (4). A better understanding of leprosy dynamics under conditions specific to high-burden countries, in hand with continued leprosy elimination activities and innovative solutions, will be necessary to advance progress in reducing the incidence of leprosy in the remaining endemic areas.

Leprosy: clinical profile, epidemiology, and risk factors

Leprosy is a chronic infectious disease caused by *Mycobacterium* (*M.) leprae*, primarily affecting the skin, nervous system, eyes, and nasal mucosa of the upper respiratory tract (5). While the exact route of bacterial transmission is yet to be completely understood, bacterial secretion via droplets from the nasal mucosa of an infected individual are considered the most important mode of transmission (6). After sustained exposure to *M. leprae*, only a subset (approximately 5%) of individuals proceeds to develop clinical disease, an outcome determined by the immunocompetence of the exposed individual (6, 7). The length of time between infection and disease varies but is believed to be shorter for paucibacillary (PB) disease (2-5 years) compared to multibacillary (MB) disease (5-10+ years) (8, 9). After the incubation period, the disease enters an indeterminate phase, characterized by various clinical symptoms that make diagnosis difficult (6, 9). A process known as polarization ultimately occurs, leading to more advanced phases of clinical disease with distinguishable symptoms and mediated by the type and strength of the host immune response (9-11). Classification of the disease into subtypes is critical for determining a suitable course of treatment and provides information necessary for research on leprosy pathophysiology (10). Major leprosy classification schemes include Madrid (1953), Ridley-Jopling (1966) and WHO (1982, 1988, 1996). The Madrid system defines four subtypes – tuberculoid (T) and lepromatous (Virchowian) (L), which are completely immunologically polarized groups; and borderline (B) and indeterminate (I). The Ridley-Jopling system further divides cases into 5 subtypes to better characterize disease classified as 'borderline' – polar tuberculoid (TT), borderline tuberculoid (BT), borderline borderline (BB), borderline lepromatous (BL), and polar lepromatous (LL). Both Madrid and Ridley-Jopling classify leprosy based on a combination of clinical, immunological, histological,

and bacteriological criteria. The most recent WHO classification (1996) was developed for the operational purposes of diagnosis and treatment decisions in the field, dividing cases into two subtypes –PB, which is roughly equivalent to TT and BT Ridley-Jopling subtypes and is defined as a case with up to five skin lesions, and MB, equivalent to BB, BL, and LL Ridley-Jopling subtypes, B and L Madrid subtypes, and defined as a case with more than five skin lesions (9, 10).

As of 2017, more than 80% of new leprosy cases occur in the three highest-burden countries – India, Brazil, and Indonesia (4). Globally, new case detection rates have slowly declined from approximately 250,000 toward 210,000 cases annually over the past 10 years. During the same time period, Brazil contributed 92.3% of leprosy cases in the Americas region (4).

Apart from exposure to *M. leprae* via contact with an infected individual, there is evidence of yet unidentified bacterial reservoirs that may contribute to leprosy transmission in endemic areas, including environmental or zoonotic reservoirs and trauma-related transmission (12, 13). Risk factors related to contact with a leprosy patient include age, disease subtype, and physical or genetic proximity (14). Many risk factor studies aim to characterize the well-established link between leprosy and poverty or other unfavorable socioeconomic conditions. Documented factors related to the socioeconomic status of cases include household crowding, poor sanitary and economic conditions, and food shortage (15). Ecological studies offer further support for the existence of associations between leprosy and poverty, poor sanitation, and crowded living conditions, though reverse causality can play a role in the associations detected by such study designs and is often undetectable (15).

Of particular relevance to this study, several risk factors are postulated to alter the host immunologic response to *M. leprae*, thus potentially affecting the development of disease or polarization into different subtypes. Risk factors identified from the literature that may have an impact on an individual's immune status and thus leprosy development or disease polarization include age, genetic predisposition, nutritional status, health status, previous BCG vaccination, and infection with soil-transmitted helminthiases (13, 16).

Since 1982, treatment for leprosy has been multi-drug therapy (MDT), a combination of dapsone, rifampicin, and (in MB cases) clofazimine, for a duration of six months to one year depending upon the disease subtype (PB or MB). Free access to MDT, which is highly effective in clearing infection with *M. leprae*, for all leprosy cases since 1995 has been an important contribution to the drastic reduction in leprosy cases globally (9).

Leprosy histopathology

Leprosy is suspected to enter the body via the nose and travel to peripheral nerves and skin via the circulation. The polarization of the host immune response to bacterial invasion is responsible for disease differentiation. High cell-mediated immunity, characterized by a strong Th1 cytokine (inferferon-γ, interleukin (IL)-2, tumor necrosis factor (TNF), and IL-15) and TH-17 response (IL-17A, IL-17F, IL-21, and IL-22), results in bacterial containment and clearance (17). The Th1-dominant response is characteristic of polar tuberculoid leprosy. Alternatively, polar lepromatous leprosy is a result of a humoral Th2 and T regulatory (Treg) immune response, characterized by increased IL-4 and IL-10, antibody production, absence of granulomas, and failure to inhibit *M. leprae*

growth (17). Borderline disease is immunologically dynamic, exhibiting mixed histopathology and progressive reduction of cell-mediated immune response toward the more severe subtypes (17). While the Th1-Th2 immune paradigm has been used for many decades to understand the histopathology of leprosy, advances in the field of immunology have revealed great complexity of the process, and continued progress in this area may create opportunities for development of new therapeutic targets for leprosy (17, 18). Current understanding of the interaction between *M. leprae* and the host indicates that the ability of the host to mount a sufficient immune response greatly influences initial bacterial growth and infection (17).

Soil-transmitted helminthiases

Soil-transmitted helminthiases (STH) are among the most common infections in the world, affecting over 24% of the world population. The main species that infect humans are roundworms (*Ascaris (A.) Lumbricoides)*, whipworms (*Trichuris (T.) Trichuria)*, and hookworms (*Necator (N.) Americanus* and *Ancylostoma (A.) Duodenale*) (19). STH infection most often occurs in tropical and sub-tropical climates, among individuals with poor access to adequate water, sanitation, and hygiene (19, 20). The highest infection intensity and prevalence of *A. lumbricoides* and *T. trichuria* is in children and adolescents (age 5-15), in contrast to hookworms, for which the main burden of disease is among adults, though intense infection can also occur among children (20, 21). STH are spread via infected individuals who shed eggs in feces, contaminating soil upon defecation. Infection of healthy individuals occurs after ingestion of eggs that attach to produce grown in contaminated soil, water sources containing eggs, or, as commonly occurs among

children, direct contact with contaminated soil and the mouth via dirty hands (19). Hookworm eggs can hatch in contaminated soil and the worm can then burrow into the skin upon contact (19).

Periodic anthelmintic treatment to all at-risk people living in areas where prevalence of infection exceeds 20%, a strategy known as mass drug administration (MDA) of preventive chemotherapy (PC), is the currently recommended strategy for controlling morbidity caused by STH infections (19). The primary aim of regular anthelmintic treatment is to reduce morbidity due to STH by reducing individual infection intensity, thus reducing population prevalence and the individual severity of health-related outcomes (20, 22). Albendazole and mebendazole, the drugs most commonly distributed for PC, have varying efficacy in clearing worm infection by species. *Ascaris lumbricoides* has the highest efficacy rate, estimated to have an egg-reduction rate (ERR) between 97.6-99.9%, followed by hookworm, with estimated ERR between 61-92.4%, and whipworm between 49.9-86.8% (22-25). Many deworming programs are focused on reaching school-age children because of high exposure levels and greater susceptibility to infection at this stage of life. Further, infection can cause developmental delays during the time of intense growth and development that occurs at young ages. Use of school infrastructure as distribution centers promotes ease of access to the population of school-age children and greatly reduces distribution costs (26). Recently, the effectiveness of school-based deworming on population-level control of STH morbidity has been questioned, with modeling estimates suggesting that in order to support long-term STH control and elimination activities, deworming programs may need to expand to larger population groups (27, 28). This is due to the reinfection that occurs rather quickly after deworming, theorized to be the result of

a persistent community reservoir (29). While PC has proven an effective strategy to reduce STH morbidity in countries with lacking infrastructure and low educational coverage, sustainable reductions in STH burden in endemic areas will also require improvements in access to clean water, sanitation, and hygiene (20, 22).

Helminthic immunomodulation

To promote survival within the human host, helminthic parasites have evolved mechanisms to regulate host immune responses (30). The immunologic tolerance exhibited by the host in response to helminth infection is characteristic of infected asymptomatic individuals, in contrast to those with pathologic manifestations of infection (30). Through the course of infection, both helminth and host-derived factors (cytokine and chemokine mimics, Treg cells, IL-10, TGF-beta, PD-1, and CTLA-4, among others) in conjunction, shift host immunity toward a Th2 profile. Consequently, Th1 and Th17 responses are downregulated, the parasite is tolerated, and immune homeostasis is maintained without significant tissue damage (30, 31). The shift toward a Th2 immune profile has been reported for hookworm, ascariasis, and whipworm infections (20, 32-34). Some studies have found evidence of a relatively quick normalization of the immune profile upon deworming (35, 36).

Immunomodulation spill-over effects

Host immune regulation in response to STH infection is thought to interfere with the host's ability to mount an appropriate immune response to other pathogens. Chronic helminth infection has been observed to affect the Th1 response necessary to prevent active

disease during infection with *Mycobacterium tuberculosis*, as well as reduce immunogenicity of BCG vaccination (36, 37). The natural development and progression of HIV and malaria, diseases of great importance to the global community, may also be altered by helminthic immunomodulation, however sufficient evidence to characterize the significance of these co-infections is lacking (38, 39). Conversely, there is strong evidence in animal models that helminthic immunomodulation may have protective effects against allergy and autoimmune disorders (40, 41). Such effects have been observed in human studies, though to a much lesser extent, necessitating more and better research to assess the true impact of helminth co-infection on diseases of global importance.

STH-leprosy co-infection studies

The effects of STH infection on immune response to *M. leprae* have been explored in a handful of studies. While there is general consensus that STH infection has a negative impact on leprosy outcomes, there is limited evidence to conclusively characterize the interaction that occurs between the two diseases. It is biologically plausible that the dominant Th2 response present during helminth infection could bias the immune response to leprosy, thus prompting STH-infected individuals to either develop symptomatic disease that may not have developed in an immunologically healthy individual, or to develop a more severe leprosy subtype than should have occurred with an appropriate immune response. Three studies (2 cross-sectional, 1 prospective cross-sectional) have found a statistically significant relationship between infection with soil-transmitted helminths and leprosy, particularly MB leprosy (16, 42, 43). One recent study, however, was published documenting an association between absence of STH infection and leprosy reaction (44).

The long incubation period associated with leprosy makes this a particularly difficult relationship to characterize; whether infection with STH or *M. leprae* occurs first in co-infected patients has not been captured by any study design so far and may provide strong evidence as to which aspect of leprosy disease development is most impacted by STH immunomodulation. Further, no studies to date have captured the process of infection with STH or *M. leprae* or the establishment of clinical disease in concurrence with established infection of the other disease. Many questions regarding STH-leprosy co-infection remain unanswered, and deeper exploration of the relationship could provide powerful insight into the pathology of both diseases, as well as offer support for important control and elimination activities.

Brazil's Neglected Tropical Diseases (NTD) Campaign

In 2011, the Brazilian Ministry of Health released an integrated plan for the elimination and control of leprosy, schistosomiasis, lymphatic filariasis, STH, onchocerciasis and trachoma (45). The aim of the plan was to improve the health of schoolchildren in all Brazil municipalities with a contemporaneously high burden of NTDs and poverty (46). Part of the strategy included a school-based deworming campaign paired with leprosy education and case finding. Children were taught the symptoms of leprosy and given self-examination forms, which they were encouraged to take home and share with their families. Upon receipt of the self-examination forms, health officials followed up suspected cases and once confirmed, contacts of cases. The campaign was implemented in over 20,000 schools across Brazil and resulted in early detection of 407 new leprosy cases (46).

Goals of this Study

Brazil's integrated NTD campaign, especially the school-based deworming that took place in specified municipalities, provides a unique opportunity to study the effects of deworming on the incidence of leprosy, indicated by the new case detection rate (NCDR), and percentage of cases that progress to the more severe leprosy subtype (MB). Using available data from Brazil's national notifiable disease surveillance system and the school-based deworming initiative, we hope to quantify the impact of the deworming intervention on leprosy new case detection rates.

Chapter II: Manuscript

Title: Leprosy in the Wake of Helminth Immunomodulation:

A study on the impact of deworming on leprosy outcomes in Vale do Rio Doce, Brazil

Cynthia N. Jones

Abstract:

Background: Helminthic immunomodulation is believed to affect the human immune response to several diseases of public health importance, including leprosy. The implementation of a four-year school-based deworming and leprosy detection campaign throughout Brazil brings about an important opportunity to study the as-yet unidentified effects of soil-transmitted helminth treatment on the incidence of leprosy and its subtypes. Methods: We conducted two analyses on incident cases of leprosy retrieved from SINAN, Brazil's nationally notifiable disease surveillance system, between 2002 and early 2017. The first was a difference-in-differences analysis in which trends in leprosy outcomes within Vale Do Rio Doce, Minas Gerais, Brazil, were compared in the years leading up to the commencement of Brazil's national school-based deworming campaign through the period during which anthelmintic treatment actively occurred in selected municipalities. Linear regression was used to compare the effect of school-based deworming on leprosy new case detection rate and percent multibacillary cases in the general population and among children. A spatial hotspot analysis was conducted and evaluated to complement the difference-in-differences models and to determine the effect of school-based deworming on inclusion in a leprosy hotspot.

Results: No significant effect of school-based anthelmintic treatment on new case detection rate, percent of multibacillary cases, or pediatric new cases and percent of multibacillary cases was observed from the aspatial difference-in-differences model. Minor changes in hotspot locations were observed throughout the study period; however, no statistically significant difference in hotspot changes between intervention and control groups was detected.

Conclusions: While no significant effect of anthelmintic treatment on leprosy incidence or percent of multibacillary cases was observed, the cluster analysis did reveal areas of consistently high leprosy transmission. These areas coincide largely with municipalities selected to take part in annual school-based deworming, presenting future opportunities for further integration of programs to eliminate and cooperatively control these diseases. Additional analyses are essential to identify the true effect of anthelmintic treatment on leprosy incidence and polarization, as well as further characterize the relationship between these diseases.

Introduction

Leprosy, also called Hansen's disease, is one of the oldest known diseases in human history (1). It primarily affects the skin, nervous system, eyes, and nasal mucosa of the upper respiratory tract, and can cause physical abnormalities or disability if left untreated (1, 5). Leprosy is caused by infection with the acid-fast bacillus *Mycobacterium (M) leprae*. As of 2017, more than 80% of new leprosy cases occur in the three highest-burden countries – India, Brazil, and Indonesia (4). Globally, new case detection rates have slowly declined from approximately 250,000 toward 210,000 cases annually over the past decade.

Brazil contributed 92.3% of leprosy cases in the Americas region during that time (4). Risk factors for leprosy include contact with an infected individual, household crowding, poor sanitary and economic conditions, and food shortage (15). Risk factors related to contact with a leprosy patient include age, disease subtype, and physical or genetic proximity (14). There is evidence of yet unidentified bacterial reservoirs that may contribute to leprosy transmission in endemic areas, including environmental or zoonotic reservoirs and trauma-related transmission (12, 13). Infection with *M. leprae* is followed by a long period of latency, followed by indeterminate disease. Over the course of infection, disease differentiation occurs, which is largely dependent on the host immune response to the infection. Polar tuberculoid leprosy, the most mild form of the disease, characterized by the presence of up to five skin lesions, is associated with high cell-mediated immunity, characterized by a strong Th1 cytokine (inferferon-γ, interleukin (IL)-2, tumor necrosis factor (TNF), and IL-15) and TH-17 response (IL-17A, IL-17F, IL-21, and IL-22). Differentiation into the most severe form, lepromatous leprosy, is characterized by a humoral Th2 and T regulatory (Treg) immune response, characterized by increased IL-4 and IL-10, antibody production, absence of granulomas, and failure to inhibit *M. leprae* growth (17). Borderline disease is immunologically dynamic, exhibiting mixed histopathology and progressive reduction of cell-mediated immune response toward the more severe subtypes (17). Several risk factors are postulated to alter the host immunologic response to *M. leprae*, thus potentially affecting the development of disease or polarization into different subtypes. Risk factors identified from the literature that may have an impact on an individual's immune status and thus leprosy development or disease polarization

include age, genetic predisposition, nutritional status, health status, previous BCG vaccination, and infection with soil-transmitted helminthiases (13, 16).

Soil-transmitted helminthiases (STH) are among the most common infections in the world. STH are spread via infected individuals who shed eggs in feces, contaminating soil upon defecation. Infection of healthy individuals occurs after ingesting eggs that attach to produce grown in contaminated soil, water sources containing eggs, or, as commonly occurs among children, direct contact with contaminated soil and the mouth via dirty hands (19). Hookworm eggs can hatch in contaminated soil and the worm can then burrow into the skin upon contact (19). To survive within the human host, helminths have evolved mechanisms to regulate host immune responses (30). The immunologic tolerance exhibited by the host in response to helminth infection is characteristic of infected asymptomatic individuals, in contrast to those with pathologic manifestations of infection (30). Through the course of infection, both helminth and host-derived factors (cytokine and chemokine mimics, Treg cells, IL-10, TGF-beta, PD-1, and CTLA-4, among others) in conjunction, shift host immunity toward a Th2 profile. Consequently, Th1 and Th17 responses are downregulated, the parasite is tolerated, and immune homeostasis is maintained without significant tissue damage (30, 31). Host immune regulation in response to STH infection is thought to interfere with the host's ability to mount an appropriate immune response to other pathogens. It is biologically plausible that the dominant Th2 response present during helminth infection could bias the immune response to leprosy, thus prompting STH-infected individuals to either develop symptomatic disease that may not have developed in an immunologically healthy individual, or to develop a

more severe leprosy subtype than should have occurred with an appropriate immune response.

In 2011, the Brazilian Ministry of Health released an integrated plan for the elimination and control of leprosy, schistosomiasis, lymphatic filariasis, STH, onchocerciasis and trachoma (45). Components of the plan included a four-year school-based deworming program implemented in municipalities where the burden of STH reached or exceeded 20%, and school-based leprosy education and case-finding. The campaign was implemented in over 20,000 schools across Brazil and resulted in early detection of 407 new leprosy cases (46). The goals of this study are to evaluate the impact of the deworming that took place between 2012 and 2016 on leprosy outcomes in the area selected to receive the interventions.

Methods

Ethical considerations: All data included in the study were anonymized prior to analysis. IRB approval was granted by the Emory University IRB review board.

Study area: The Doce River Valley [Vale Do Rio Doce], the Easternmost Central mesoregion of Minas Gerais, Brazil, was chosen as the study site. The units of analysis were the 102 municipalities located within the Doce River Valley. Of the municipalities analyzed, 45 were selected to participate in annual school-based deworming as part of the Brazilian Ministry of Health's "Integrated Strategic Action Plan for elimination of leprosy, lymphatic filariasis, onchocerciasis, schistosomiasis, trachoma as a cause of blindness and control of soil-transmitted helminthiases (STH)" [Plano Integrado de Ações Estratégicas

de Eliminação da Hanseníase, Filariose, Esquistossomose e Oncocercose Como Problema de Saúde Pública, Tracoma Como Causa de Cegueira e Controle das Geohelmintíases]. The Doce River Valley, a 41,809 km^2 area, is covered by a seasonal semideciduous forest, part of the Mata Atlantica biome (47, 48). The climate is hot and humid, with 4 to 5 months of dry season. The estimated 2017 population of the Vale Do Rio Doce area is 1,719,096, nearly 50% of whom live in the five largest municipalities (Governador Valardes, Ipatinga, Coronel Fabriciano, Caratinga, and Timoteo). Priority economic inputs for Vale do Rio Doce are mining and agriculture (47, 48).

Study Population: The study population consisted of all residents of the Vale do Rio Doce area, and leprosy cases were defined as those newly diagnosed and reported between April 01, 2002 and April 01, 2017 within the region of Vale Do Rio Doce. The unit of analysis was the municipality.

Data Sources and Methods: Data on newly detected cases of leprosy were retrieved from Brazil's Information System for Nationally Notifiable Diseases [Sistema de Informacao de Agravos de Notificacao] (SINAN), for the years 2002 to early 2017 in the State of Minas Gerais, Brazil. These data were limited to cases associated with residence in one of the 102 municipalities in the Vale do Rio Doce area. In Brazil, leprosy is a nationally notifiable disease. Upon diagnosis of a case, healthcare providers enter demographic information including age, sex, race, housing type, and education level, along with Madrid and WHO disease classification, bacilloscopic results, disability grade, assigned treatment, and updates on treatment and care into a database. A line listing of municipalities in the state

of Minas Gerais identifying the locations of school-based deworming and leprosy screening campaigns by year was presented by the Minas Gerais Department of Health. Data on socioeconomic indicators such as poverty, the municipal human development index, and life expectancy at birth for each municipality was collected from the Atlas of Human Development in Brazil [Atlas do Desenvolvimento Humano no Brasil] (49). Information on sanitation, population estimates, and territory divisions were extracted from the Brazilian Institute of Geography and Statistics [Instituto Brasiliero de Geografia a Estatistica] (IBGE) website and the Atlas of Human Development in Brazil (48, 49).

Analysis periods were developed with respect to the implementation dates of the deworming program. Annual data were summarized into adjusted analysis years, beginning on April 1 and ending on March 31 of the next calendar year, and the use of adjusted years in the text is indicated by an (*). During the aspatial analysis period (04.01.2002 - 04.01.2017), a total of 7,593 residents of Vale do Rio Doce were diagnosed with leprosy. Of these, 2,518 (33.2% of total) cases were reported during the spatial analysis period (04.01.2009-04.01.2017).

Aspatial analysis: Cases reported during the study period were aggregated by month and year of diagnosis and municipality of residence. Descriptive statistics of cases stratified by control and intervention group were calculated using χ^2, t-tests, or Wilcoxon signed-rank tests. Exchangeability of the intervention and control groups was assessed across demographic and social equality indicators, however, due to the pre-existent (non-randomized) nature of the intervention, true exchangeability is unachievable under the conditions of the study. Time trends of the outcomes of interest were visualized and

stratified by intervention and control group. A difference-in-differences (DID) method was used compare the change in outcomes between the control group (57 municipalities in which schoolchildren did not receive anthelmintic treatment) and the intervention group (45 municipalities in which annual deworming occurred). A linear model was used for NCDR outcomes. For percent multibacillary outcomes, a beta regression model was used. To regress percent MB cases, every municipality that did not have an incident case of leprosy in a given year necessarily was excluded to avoid the issue of having NAs in the denominator. The regressions used to identify the effect attributable to deworming incorporated state- and time- fixed effects to control for space- and time- varying factors. Outcome variables were cube-root transformed to enhance model fit and better estimate β coefficients. Cube-root transformation was chosen to reduce right-skewness in the outcome data as it can be applied to "0" values. Robust standard errors were used to account for clustering by time and space. The equation for the main model that was used with total case and pediatric subset data was as follows:

$$Y_{gt} = \alpha + \beta_g + \beta_y + \beta_i + \beta_t + \gamma D_{it} + \varepsilon_{gt} \quad (1)$$

Where Y is the outcome of interest (NCDR or % MB), β_g refers to group-fixed effects, β_y refers to time-fixed effects, β_i is a dichotomous variable equal to 1 in municipalities selected to receive treatment, β_t is a dichotomous variable equal to 1 in time periods specified as treatment years, and γD_{it} is the interaction effect between time and intervention, which equals one in municipalities that received deworming during a year of deworming implementation. To test for violation of the parallel trends assumption, two separate techniques were used. Pre-intervention trends in cube-root transformed new case detection rate and percent multibacillary cases were assessed visually using time-trend

graphs. Overall trends were tested by nesting state-specific time trends into the models and running partial F-tests to check for significant effects attributable to state-specific time trends (50). Migration occurring in response to the intervention was eliminated as potential confounder due to lack of evidence that this would plausibly occur. Exogeneity of the outcome was assumed as the locations receiving intervention were determined based on STH endemicity levels (equal or greater to 20%), and not on leprosy distribution. There is no known association between albendazole consumption and decreased risk of leprosy, thus leprosy incidence should have no influence on STH treatment distribution. Potential confounders of the association are mainly socioeconomic in nature, and due to lack of temporal trend data in socioeconomic indicators, were not included in the models. However, the occurrence of socioeconomic shocks in any municipality over the study period, affecting both leprosy incidence and STH prevalence, would have effects that could bias model estimates. Along with the main analysis, two sub-analyses were performed to better characterize the association between the treatment and leprosy outcomes among the total study population and among the pediatric study population alone. In the first, the data was limited to the years 2009 to early 2017 to determine whether the exponential decay in leprosy incidence that occurred in the early years of the study window affected estimation of the model outside of state and time trends. To check the robustness of the model to the overall time trend, the study period was subset to later years that demonstrated a lower rate of change across all units. A second sub-analysis model was run excluding the 34 treatment group municipalities that did not dispense deworming treatments for all four years (2013-2016). This model demonstrated whether satisfaction of the irreversibility of treatment assumption would discernibly change the model estimates. The sub-analysis accounted for

this gap in treatment by removing groups from the treatment group that did not complete distribution of deworming treatments for all four treatment years. This model was expected to recover any masked treatment effect due to prolonged helminth reinfection. A robustness model was fit using a false date of treatment (2009*) to test the model's capability of identifying a treatment effect. DID was conducted for four outcomes of interest altogether. The software used for statistical analysis and graphical visualization was R version 3.5.2 (Vienna, Austria). R packages used for analysis are listed in Appendix Table 2.

Spatial Analysis: Cases reported during the study period were aggregated by year and municipality of residence and visualized in a raw incidence rate map. Spatial empirical Bayesian rate smoothing was used with a queen contiguity weights matrix to account for small population sizes in several municipalities. Both maps were visually compared to a map of the area selected for intervention. Global Moran's I statistic was run to identify the presence of global clustering, and results were randomized 999 times to assess significance. To identify the locations of clusters, Local Getis-Ord statistic (Gi*) was run with 999 permutations. Different significance level cut-offs were tested to determine an appropriate value, and significance at the level of alpha = 0.05 was chosen as it proved useful for exploring cluster time trends. Time trends of the spatial outcomes of interest were also visualized graphically, stratified by intervention and control group. Initially, the hotspot data was fed into a DID model excluding state-fixed effects. The parallel trends assumption was evaluated visually by time trend graphs of pre-intervention outcomes. To better assess changes in hotspots prior to and during the intervention, logistic regression,

generalized linear mixed modeling, and spatial regression were performed in sequence, after checking each model for global spatial autocorrelation of the residuals, to determine the appropriate model for intervention estimation that could account for spatial structure within the data. Spatial analysis was conducted in GeoDa version 1.12.1.131 and SpaceStat version 4.0.21 (BioMedware, Anne Arbor, MI, USA), and results were visualized in ArcMap version 10.6 (ESRI, Redlands, CA, USA).

Results

Description of cases: During the study period, the average annual NCDR of leprosy across all study regions was 30.1 per 100,000 population. Among the intervention group, the average annual NCDR was 41.4 per 100,000 population, and among the control group average annual NCDR was 21.1 per 100,000 population. Demographic characteristics of cases stratified by intervention group are reported in Table 1. All demographic characteristics of cases were statistically significantly different between the control and intervention groups at an alpha level of 0.05. Educational status was lower and leprosy disability grade higher within the control group. Within both intervention and control groups, the age category with the highest case count was 45-54; however, the overall age structure of cases significantly differed between groups. The mean age of all cases was 44.25 years. Cases in the intervention group were from urban areas more often than those in the control group, and cases were less often men in the intervention group compared to the control group. When limited to MB cases, sex and age differences between the intervention and control groups were not significant, and race, education level, residence location, and disability grade were significantly different at the level of alpha = 0.05. Due

to the differences in demographic characteristics of cases by intervention group, an exploratory analysis of sociodemographic differences between control and intervention groups was performed at the level of the municipality and results are presented in Table 2.

Description of municipalities: Table 2 contains the results of the municipality analysis. Variable descriptions for Table 2 are listed in Appendix Table 1. The intervention group had statistically significantly higher poverty and mortality rates, lower access to adequate sanitation, and a higher GINI score than the control group. In contrast, the control group had statistically significantly longer life expectancy and a higher human development index score than the intervention group. The overall age structure between the control and intervention groups was not statistically significantly different.

Aspatial analysis: The full results of the difference-in-differences analyses and sub-analyses are listed in Tables 3-9.

The difference-in-differences model was used to determine the effects of the treatment (deworming and leprosy education and screening) on four separate outcomes (total NCDR, percent (proportion) MB of cases, total pediatric NCDR, and pediatric percent MB of cases). Prior to variable transformation, the outcome variables each appear to have followed similar trends stratified on intervention and control group, as conveyed in Figures 1-4. Confounders were controlled for by including fixed effects for both group and time. The results of the main model for all four outcomes are presented in Table 3. All four full models (Models 1.1-4.1) were lightly predictive of outcome trends ($0.25 < R^2 < 0.46$), though the intervention itself did not have a statistically significant effect on the

outcomes. Intervention estimates for the full models ranged from -0.32 to 0.02, with relatively narrow confidence intervals that incorporated the null. Regarding overall NCDR and proportion MB, the intervention had approximately no effect. Among the pediatric population, however, the intervention had a negative relationship with both NCDR and proportion of multibacillary cases. The estimate associated with pediatric NCDR was -0.17 (-0.41, 0.07), which approached, but did not attain, statistical significance. The second set of models (Models 1.2, 3.2) were statistical tests of the parallel trends assumption for NCDR and pediatric NCDR. Partial F-tests comparing models 1.1 and 3.1 with their state-specific time trend-nested counterparts were not statistically significant at an alpha level of 0.05, confirming that the models met the parallel trends assumption. State-specific time trends were not nested in the beta regression models for proportion multibacillary cases due to the large number of municipalities that experienced either extremely high or low proportions of multibacillary disease, thus optimization parameters for nested models were unavailable. Visual evaluation of time trends for each leprosy outcome (Figures 5-8) provides further evidence that the parallel trends assumption is not violated for NCDR (Figure 5) or pediatric NCDR (Figure 7) and offers strong evidence that the assumption is not violated for pediatric proportion of multibacillary cases (Figure 8). The time trend graph of the total proportion of multibacillary cases (Figure 6) is not as clearly parallel as the other graphs.

The first set of sub-analysis models, for which data were limited to the years 2009 to early 2017, estimated non-significant negative relationships of the intervention on outcomes for all four models (Models 1.3-4.3). The model for total NCDR resulted in an estimated treatment effect of -0.15 (-0.46, 0.16). Total proportion MB, pediatric NCDR,

and pediatric proportion MB resulted in similarly negative non-significant estimates with relatively small confidence intervals. The nested state-specific time trend models exposed violation of the parallel trends assumption for pediatric NCDR (Models 1.4, 3.4). Pediatric proportion of multibacillary cases is the only graph that strongly upholds the parallel trends assumption for the years 2009-2012 (Figure 8). Of the others, total NCDR slightly violates the assumption (Figure 5) and pediatric NCDR (Figure 7) and total proportion of multibacillary cases (Figure 6) do not satisfy the assumption.

The models for the final sub-analysis, based on a subset of the original data including only intervention municipalities that completed four consecutive years of deworming, resulted in non-significant treatment effects, as well (Models 1.5-4.5); however, for both total NCDR and pediatric NCDR, the parallel trends assumption was violated according to the significance of the partial F-test assessing the nested state-specific time trends (Models 1.6, 3.6). Visualization was deemed unnecessary due to the sparseness of the data after subsetting.

Lastly, a robustness check was conducted. Because no model estimates returned significant values, the check for robustness was not necessarily informative to indicate the sensitivity of the model to intervention effects, but rather was carried out for the purpose of completeness of model evaluation practice. To check model robustness, a false intervention start date (2009) was inserted into the model in place of the true intervention start date. All estimates returned positive non-significant values except for pediatric NCDR, which yielded an intervention estimate of -0.24 (-0.43, -0.05) (Models 1.7-4.7). A nested state-specific time trends check for parallel trends revealed that the pediatric NCDR model did not violate the parallel trends assumption.

Spatial Analysis: Visual interpretation of the panel data indicates a gradually decreasing leprosy (raw and smoothed) NCDR trend within the study area over the entire study period (Figures 9 and 10); however, a few municipalities maintained a high NCDR. The area selected for deworming appears to greatly overlap with areas of ongoing leprosy transmission (Figure 11). The time trend for inclusion in each hotspot category (smoothed versus raw and cluster versus core), as presented in Figures 12-15, reveals similarly decreasing trends during the intervention period for both groups; however, the intervention group appears to have experienced a more rapid decrease across all outcome categories. The modified difference-in-differences analyses (excluding state-fixed effects and higher-order variables based on them) used to determine the intervention effect on inclusion in a leprosy hotspot, as determined by global and local clustering tests (Table 10, Figures 16 and 17), indicated that the intervention did have a weakly negative effect on spatial hotspot outcomes (Table 11). The smoothed rate cluster model was the only one for which the intervention effect just reached statistical significance at -0.11 (-0.20, -0.03). Nested models of state-specific time trends were not used for the models due to the presence of a spatial aspect in the outcome variable. Interpretation of the graphical time trends indicated that the parallel trend was not violated for the models (Figures 18-21). However, further analysis was necessary to address the spatial component of the model. Aspatial linear regressions were fit for each outcome and all were rejected on the criteria that residuals were highly autocorrelated. GLMMs with random intercepts were fit next and determined to be sufficient to provide valid estimates for cluster (not cluster core) outcomes due to the lack of residual spatial autocorrelation. GLMM estimates of the effect of deworming on

hotspot outcomes were negative, with the smoothed rate model reaching significance -0.99 (-1.94, -0.03). Cluster core outcomes had residual correlation after fitting the random intercept model. Spatial regression was run and the presence of spatial lag identified. The results of the analysis are presented in Table 12.

Discussion

The statistically significant differences present between cases belonging to the intervention and control groups as well as those between intervention and control municipalities are indicative of differing disease environments, though statistically significant differences between control and intervention municipalities should be interpreted with caution due to the relatively small sample size (N = 102: intervention = 45, control = 57). While more overall cases and higher incidence were detected in the intervention areas, they less often presented with grade two disability upon diagnosis, indicating that earlier case detection may occur in the intervention areas. The higher proportions of male and older cases observed from control areas may be further suggestive of late case detection in these areas. The majority of cases in both areas were associated with urban living environments, though more so within the intervention group. While case detection may appear higher in the intervention group, municipal social conditions appear somewhat worse in these areas. Higher poverty and mortality rates, a higher GINI score, and lower access to adequate sanitation indicate that municipalities in the intervention group may be less equitable with worse living conditions for the poor. Non-random assignment of the intervention was expected to result in baseline differences between the control and intervention groups. While difference-in-differences is considered robust to

non-exchangeability by controlling for state effects, quantifying observed differences between the groups can provide better insights for interpretation of model effect estimates.

Regarding the DID models, two assumptions underlie the credibility of the difference-in-differences analysis. The first is what is referred to as "strict exogeneity" in econometrics. That is, the time of intervention implementation must not be related to factors driving outcome development. The integration of leprosy case finding activities into the same program as deworming makes this relationship unclear. However, it appears that the municipalities designated to participate in deworming were selected on the basis of community worm burden, as stated in the integrated action plan from the Ministry of Health. Thus, while combining interventions may have influenced leprosy NCDR, it is still reasonable to assume leprosy determinants did not bias the selection of locations for deworming. Fortunately, the change in NCDR attributable to the program was quantified as 407 new cases (293 schoolchildren and 114 additional case contacts), offering insight as to the true impact of the concurrent leprosy case finding activities on leprosy outcomes. The second assumption underpinning the validity of the design is that important unmeasured variables are either time-invariant group attributes or group-invariant time attributes and can thus be controlled for in a model by incorporation of group and time fixed effects. This is referred to as the parallel trends assumption and is difficult to prove. The use of two assumption checks (graphical interpretation and nested state-specific time trend models) enhanced credibility of the estimates from models that met the assumption in both areas.

The results of the aspatial models indicate that the intervention may have had a small, though insignificant, negative effect on NCDR and proportion of multibacillary

cases. The models did not optimally fit the data, in part due to the high rate of zeros across time and state- a problem that can greatly increase the complexity of model estimation. While the main effect of interest was the school-based deworming treatment, the effects captured as intervention estimates necessarily include the simultaneous effect of leprosy education and screening, an effect that certainly increased the NCDR during the study period. There is a strong possibility that the observed deworming effects are mitigated by the concurrent increase in reported cases (407 - 293 children and 114 adults throughout Brazil) (46). Pediatric NCDR is especially low, so any amount of pediatric cases identified by the program likely mitigated the observable effects of anthelmintic treatment on leprosy outcomes in the group hypothesized to be most positively impacted by a deworming initiative. Even so, the largest intervention effects identified in the models were with respect to pediatric outcomes, as expected due to the direct deworming effects on children. With a much smaller sample size than the total population, especially regarding pediatric proportion of multibacillary outcomes, pediatric estimates were very unstable with wide confidence intervals. A problem occurring with several of the models was that upon subsetting, the parallel trends assumption was more difficult to satisfy. This is likely due to the higher weight given to each observation as a result of limiting the observations considered, reducing model robustness to random spikes or dips in the outcomes. Finally, the robustness check for which the model was given a false intervention start date revealed a significant difference in pediatric NCDR that met model assumptions. Exploring the graphical trend revealed a spike in pediatric NCDR occurring in 2008 that appears to have more greatly impacted the intervention groups, thus leading to a significant difference in the decreasing NCDR trend in following years. This may be indicative of events around

2009 that affected pediatric case detection differentially between intervention and control groups. Without knowing details of the history of leprosy control within the Doce River Valley, it is difficult to hypothesize what drove the spike in cases as well as the subsequent drop.

The spatial models, which incorporated a neighbor component into estimates, were useful in visualizing the NCDR trend heterogeneity. Comparison of the raw and smoothed NCDR maps (Figures 9 and 10), to the areas selected for deworming (Figure 11) reveals the high overlap in areas most affected by leprosy and STH. The hotspot maps (Figures 16 and 17) indicate one continuously hot area of high leprosy transmission in the east, with borders that shift each year. Those municipalities that shift between inclusion and exclusion in a hotspot offer a unique future opportunity to study temporal changes in drivers of leprosy and subsequent changes in leprosy outcomes. When placed into a DID model, the only outcome that deworming had a significant effect on was membership in a smoothed leprosy cluster. However, acknowledging the inherent spatial dependence in the definition of a hotspot, models that accounted for this were considered necessary to determine the validity of this estimate. Aspatial logistic regression resulted in poor model fit for all four outcomes. The residuals of the aspatial models were highly globally correlated, thus a GLMM was fit with random intercepts for municipalities. For membership in either a raw or smoothed cluster, the GLMMs performed well and residuals were not spatially correlated. For cluster cores, a spatial regression was run, and spatial lag was identified in the model, indicating that both observations and their residual errors were correlated. However, the GLMM results revealed that the intervention had a statistically significant effect on inclusion in a smoothed hotspot (-0.99 [-0.03, -1.94]).

This could be a true effect, in agreement with the overall negative effects observed in the aspatial models, and in strong agreement with the DID estimate of the same outcome. It is possible, however, that spatial smoothing overestimated the NCDR in certain areas, leading to false municipality inclusion in a hotspot. Interpretation of the intervention effects must be done in the context of the outcomes being explained and any transformations that occurred.

Limitations of this study include the lack of a generalizable modeling method with which to apply difference-in-differences to a number of varied outcome types (proportion, rate, and dichotomous). Literature regarding proper methods for implementation of the technique in the context of public health is sparse, and there is a great need for reproducible examples and further exploration of this useful method. Another limitation was that the time to immune system rebound after deworming was estimated to be approximately two weeks, an optimistic assumption that could have significant effects on model estimates. There are no published estimates for the length of time to immune system rebound after deworming, but there is strong evidence that immune rebound has occurred within six months of treatment. It is plausible that the immune recovery effect was not yet apparent during the time period chosen for the study. Further, the incompleteness of SINAN surveillance data, as was found in a study by Filho et al (52), is also a concern, as underreporting, especially if unevenly distributed between intervention and control groups, would certainly bias the estimate of the effect. The subtypes of leprosy are often misclassified, a data quality issue that has been quantified by assessing agreement between available classification methods (53). A difference in late case detection between the intervention and control groups is evident in the data, as indicated by the disability grade

of cases entered into SINAN, and it is also a threat to the validity of the design, as diagnosis and reporting of a new case after prolonged disease affects observed incidence rates, thus making identification of onset-related factors more difficult.

The combination of spatial and aspatial methods to analyze and interpret relationships of interest can result in stronger analyses as one method may offer insight toward the blind spots of the other method. While most results from both the spatial and aspatial components of analysis agreed but were non-significant, they provide new information on the spatial and temporal dynamics of leprosy transmission in Vale Do Rio Doce, which can be used as a starting point for further research. The minimal but negative intervention effects observed suggest that population-level deworming, such as that being undertaken in India for the DeWorm3 study (54), may have a more potent effect on leprosy outcomes, providing another ideal opportunity to further study this relationship. Difference-in-differences, having roots in economics, is a strong tool for the evaluation of programmatic impacts when a randomized controlled trial is not an option due to ethical or other hindrances. Public health intervention evaluation is a regular need in the field, and the adoption of strong methods from other areas of statistical analysis is necessary to break new ground and strengthen public health analytics. Further research should aim for better quantifying the effect of deworming on leprosy incidence, incorporating better-supported biological estimates of the immune effects of deworming as they become available.

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Tables

Table 1. Descriptive characteristics of HD cases by intervention categorization, Vale Do Rio Doce, 2002/2009 - 2016							
All cases	Total		Intervention = 1		Intervention = 0		
	n (mean)	% (sd)	n (mean)	% (sd)	n (mean)	% (sd)	
Sex							P < 0.05
M	3,749	49	2,350	47	1399	54	
Age							P < 0.05
(0-14)	557	7	449	9	108	4	
(15-24)	837	11	595	12	242	9	
(25-34)	1,059	14	690	14	369	14	
(35-44)	1,214	16	780	16	434	17	
(45-54)	1,445	19	918	18	527	21	
(55-64)	1,248	17	814	16	434	17	
(66+)	1,199	16	744	15	455	18	
Age (cont.)	44.25	19.35	43.14	19.69	46.40	18.50	P < 0.05
Race							P < 0.05
White	2,583	38	1,712	37	871	41	
Black	1,162	17	812	17	350	16	
Asian	145	2	124	3	21	1	
Mixed	2,870	42	1,997	43	873	41	
Indigenous	17	0	9	0	8	0	
Zone							P < 0.05
Urban	5,823	85	4,118	87	1705	80	
Semi-urban	63	1	34	1	29	1	
Rural	959	14	564	12	395	19	
Education level							P < 0.05
Illiterate	984	17	641	15	343	20	
Incomplete primary school	3,440	58	2,403	58	1037	60	
Incomplete secondary school	875	15	643	15	232	14	

Completed secondary school or higher	583	10	478	11	105	6	
Disability Grade							P < 0.05
0	5,134	69	3,659	74	1475	60	
1	1,784	24	991	20	793	32	
2	493	7	295	6	198	8	
MB	Total		Intervention = 1		Intervention = 0		
	n (mean)	% (s.d.)	n (mean)	% (s.d.)	n (mean)	% (s.d.)	
Sex							P = 0.28
M	2,373	60	1,956	60	417	59	
F	1,607	40	1,319	40	288	41	
Age							P = 0.06
(0-14)	108	27	71	3	37	2	
(15-24)	336	8	209	9	127	8	
(25-34)	524	13	290	13	234	14	
(35-44)	637	16	377	16	260	16	
(45-54)	784	20	424	19	360	22	
(55-64)	749	19	434	19	315	19	
(66+)	821	21	481	21	340	20	
Age (cont.)	48.51	18.23	48.27	18.50	48.84	17.85	P = 0.33
Race							P < 0.05
White	1,303	37	734	34	569	41	
Black	624	18	395	18	229	16	
Asian	82	2	68	3	14	1	
Mixed race	1,501	43	933	44	568	41	
Indigenous	14	0	6	0	8	1	
Zone							P < 0.05
Urban	2,907	73	1,788	78	1119	66	
Semi-urban	41	1	19	1	22	1	
Rural	591	15	335	15	256	13	
Education level							P < 0.05

Illiterate	682	22	425	22	257	23	
Incomplete primary school	1,793	59	1,113	58	680	61	
Incomplete secondary school	350	12	223	12	127	11	
Completed secondary school or higher	207	7	149	8	58	5	
Disability Grade							P < 0.05
0	1,946	51	1,201	53	745	47	
1	1,434	37	778	34	656	41	
2	462	12	277	12	185	12	

Table 2. Characteristics of Municipalities by intervention group (2010 values)

	Total		Intervention = 1		Intervention = 0		
	mean	s.d.	mean	s.d.	Mean	s.d.	
Male	49.78	0.91	49.65	0.99	49.88	0.85	P=0.25
% 0-14	25.07	2.23	25.75	2.26	24.54	2.07	P<0.05
% 15-24	17.51	1.34	17.66	1.31	17.39	1.33	P=0.35
% 25-34	14.66	1.34	14.3	1.14	14.95	1.42	P=0.05
%35-44	13.12	0.93	12.92	0.99	13.28	0.86	P=0.08
% 45-54	11.4	1.03	11.17	1.01	11.59	1.02	P=0.06
% 55-64	8.37	1.01	8.34	0.92	8.4	1.08	P=0.77
% 65+	13.6	2.21	13.62	1.75	13.59	2.54	P=0.92
Fertility rate	2.09	0.30	2.20	0.28	2.01	0.30	P<0.05
HH density	20.09	4.77	20.88	5.49	19.48	4.06	P=0.17
Child poverty	38.08	10.39	41	10.57	35.78	9.73	P<0.05
Poverty	23.72	7.7	26.08	8.02	21.86	6.95	P<0.05
Child indigence	13.44	6.68	15.48	7.3	11.83	5.7	P<0.05
Indigence	8.65	4.64	9.98	5.23	7.6	3.85	P<0.05
Municipal Human Development Index	0.64	0.04	0.63	0.03	0.65	0.04	P<0.05
Inadequate access to clean water and proper sewage	3.86	5.04	5.09	5.67	2.89	4.29	P<0.05

Under 1 mortality	18.04	2.67	18.61	2.4	17.58	2.79	P<0.05
Under 5 mortality	20.97	3.09	21.64	2.79	20.43	3.24	P<0.05
Life expectancy	73.4	1.57	73.04	1.36	73.69	1.67	P<0.05
GINI (income distribution)	0.48	0.04	0.5	0.04	0.47	0.04	P<0.05
Median income	666.7	177.97	654.4	157.19	676.4	193.64	P=0.88
Individuals vulnerable to poverty that travel > 1 hour to work	1.52	1.56	1.45	1.44	1.58	1.66	P=0.48

Table 3. Difference-in-differences estimates				
$Y_{gt} = \alpha + \beta_g + \beta_y + \beta_i + \beta_t + \gamma D_{it} + \varepsilon_{gt}$				
Model	1.1	2.1	3.1	4.1
Outcome	$NCDR^{1/3}$	Proportion $MB^{1/3}$	Ped $NCDR^{1/3}$	Ped Proportion $MB^{1/3}$
Deworming estimate	0.02 (-0.22, 0.27)	-0.03 (-0.40, 0.34)	-0.17 (-0.41, 0.07)	-0.32 (-2.15, 1.50)
State effects	YES	YES	YES	YES
Year effects	YES	YES	YES	YES
Adjusted R^2	0.46	0.30	0.25	0.37
Observations	1530	925	1530	925
F	13.61	-	5.84	-
Phi (beta reg)	-	1.31 (1.11, 1.50)	-	1.68 (0.82, 2.56)
DF	1,425	104	1,425	62
P	<0.05	<0.05	<0.05	<0.05

Table 4. Evaluation of the Common Trends Assumption				
$Y_{gt} = \alpha + \beta_g + \beta_y + \beta_i + \beta_t + \gamma D_{it} + \gamma_{gy} + \varepsilon_{gt}$				
Model	1.2	2.2	3.2	4.2
Outcome	$NCDR^{1/3}$	-	Ped $NCDR^{1/3}$	-
Deworming estimate	-0.22 (-0.70, 0.26)	-	0.06 (-0.31, 0.44)	-
State effects	YES	-	YES	-
Year effects	YES	-	YES	-
State-specific time trends	YES	-	YES	-
Adjusted R^2	0.47	-	0.25	-

Observations	1,530	-	1,530	-
F	7.63	-	3.51	-
DF	1,324	-	1,324	-
P	< 0.05	-	<0.05	-
Partial F test P	0.06	-	0.29	-

Table 5. Sensitivity analysis sub-setting entire dataset to years 2009-2016*				
$Y_{gt} = \alpha + \beta_g + \beta_y + \beta_i + \beta_t + \gamma D_{it} + \varepsilon_{gt}$				
Model	1.3	2.3	3.3	4.3
Outcome	$NCDR^{1/3}$	Proportion $MB^{1/3}$	Ped $NCDR^{1/3}$	Ped Proportion $MB^{1/3}$
Deworming estimate	-0.15 (-0.46, 0.16)	-0.30 (-0.79, 0.19)	-0.04 (-0.36, 0.28)	-0.32 (-2.15, 1.50)
State effects	YES	YES	YES	YES
Year effects	YES	YES	YES	YES
Adjusted R^2	0.44	0.33	0.18	0.56
Observations	816	438	816	66
F	7.26	-	2.76	-
Phi (beta reg)	-	1.35 (1.14, 1.56)	-	1.68 (0.80, 2.56)
DF	711	92	711	35
P	< 0.05	<0.05	<0.05	<0.05

Table 6. Evaluation of the Common Trends Assumption for 2009-2016* dataset				
$Y_{gt} = \alpha + \beta_g + \beta_y + \beta_i + \beta_t + \gamma D_{it} + \gamma_{gy} + \varepsilon_{gt}$				
Model	1.4	2.4	3.4	4.4
Outcome	$NCDR^{1/3}$	-	Ped $NCDR^{1/3}$	-
Deworming estimate	0.43 (-0.30, 1.16)	-	0.11 (-0.28, 0.51)	-
State effects	YES	-	YES	-
Year effects	YES	-	YES	-
State-specific time trend	YES	-	YES	-
Adjusted R^2	0.46	-	0.27	-
Observations	816	-	816	-
F	4.45	-	2.45	-
DF	610	-	610	-
P	< 0.05	-	<0.05	-
Partial F test P	0.05	-	<0.05	-

Table 7. Sensitivity analysis censoring groups without all four tx years
$Y_{gt} = \alpha + \beta_g + \beta_y + \beta_i + \beta_t + \gamma D_{it} + \varepsilon_{gt}$

Model	1.5	2.5	3.5	4.5
Outcome	$NCDR^{1/3}$	Proportion $MB^{1/3}$	Ped $NCDR^{1/3}$	Ped Proportion $MB^{1/3}$
Deworming estimate	-0.02 (-0.25, 0.20)	0.33 (-0.13, 0.78)	-0.27 (-0.72, 0.17)	0.63 (-0.75, 2.00)
State effects	YES	YES	YES	YES
Year effects	YES	YES	YES	YES
Adjusted R^2	0.45	0.31	0.24	0.36
Observations	1,155	718	1,155	137
F	13	-	5.55	-
Phi	-	1.32 (1.09, 1.55)	-	1.05 (0.80, 1.31)
DF	1,075	80	1,075	47
P	<0.05	<0.05	<0.05	<0.05

Table 8. Evaluation of the Common Trends Assumption for censored groups				
$Y_{gt} = \alpha + \beta_g + \beta_y + \beta_i + \beta_t + \gamma D_{it} + \gamma_{gy} + \varepsilon_{gt}$				
Model	1.6	2.6	3.6	4.6
Outcome	$NCDR^{1/3}$	-	Ped $NCDR^{1/3}$	-
Deworming estimate (timeint2)	-0.23 (-0.77, 0.31)	-	0.23 (-0.56, 1.02)	-
State effects	YES	-	YES	-
Year effects	YES	-	YES	-
State specific time trends	YES	-	YES	-
Adjusted R^2	0.46	-	0.25	-
Observations	1,155	-	1,155	-
F	7.43	-	3.54	-
DF	999	-	999	-
P	<0.05	-	<0.05	-
Partial F test P	<0.05	-	<0.05	-

Table 9. Robustness check				
$Y_{gt} = \alpha + \beta_g + \beta_y + \beta_i + \beta_t + \gamma D_{it} + \varepsilon_{gt}$				
Model	1.7	2.7	3.7	4.7
	$NCDR^{1/3}$	Proportion $MB^{1/3}$	Total Ped $NCDR^{1/3}$	Ped Proportion $MB^{1/3}$
Deworming	0.16 (-0.12, 0.45)	0.30 (-0.04, 0.63)	-0.24 (-0.43, -0.05)*	0.40 (-0.42, 1.22)
State effects	YES	YES	YES	YES
Year effects	YES	YES	YES	YES

Adjusted R^2	0.46	0.30	0.25	0.36
Observations	1,530	925	1,530	191
F	13.61	-	5.85	-
Phi	-	1.31 (1.11, 1.51)	-	0.93 (0.73, 1.14)
DF	1,425	104	1,425	62
P	<0.05	<0.05	<0.05	<0.05

*Using Pseudo-intervention date at year 2009

Table 10. Results of the global Moran's I Test for Global Clustering

Global Moran's I: Raw Rates				**Global Moran's I: Smoothed Rates**			
Year:	**I:**	**pseudo-p value:**	**z-value**	**Year:**	**I:**	**pseudo-p value:**	**z-value**
2009	0.4212	0.001	6.9719	2009	0.7858	0.001	13.0275
2010	0.5412	0.001	9.544	2010	0.7035	0.001	12.5051
2011	0.1907	0.004	3.4911	2011	0.5994	0.001	10.0792
2012	0.2261	0.004	3.789	2012	0.6769	0.001	11.1295
2013	0.4706	0.001	7.825	2013	0.7351	0.001	12.1697
2014	0.2429	0.001	4.2128	2014	0.6406	0.001	10.4885
2015	0.1989	0.003	3.4581	2015	0.4555	0.001	7.7518
2016	0.0377	0.085	1.5446	2016	0.07	0.01	3.23
Pediatric Global Moran's I Raw Rates				**Pediatric Global Moran's I: Smoothed Rates**			
Year	**I:**	**pseudo-p value**	**z-value**	**Year**	**I:**	**pseudo-p value**	**z-value**
2009	0.1346	0.028	2.7411	2009	0.5625	0.001	9.6085
2010	0.3106	0.002	5.3589	2010	0.6182	0.001	10.8654
2011	0.0001	0.278	0.2112	2011	0.7559	0.001	12.4924
2012	-0.0366	0.411	-0.4574	2012	0.7226	0.001	12.3974
2013	0.0164	0.204	0.5278	2013	0.6067	0.001	10.0758
2014	-0.0579	0.148	-0.8012	2014	0.392	0.001	6.8707
2015	-0.0232	0.37	-0.3649	2015	0.1094	0.015	3.319
2016	-0.0257	0.382	-0.424	2016	0.0783	0.001	2.6982

Table 11. Spatial analysis test for significant intervention effect

$$Y = \alpha + \beta_y + \beta_i + \beta_t + \gamma D_{it} + \varepsilon_{gt}$$

Model input	Raw NCDR	Raw NCDR	Smooth NCDR	Smooth NCDR
Outcome	Membership in a cluster	Membership in a cluster core	Membership in a cluster	Membership in a cluster core
Deworming estimate	-0.15 (-0.31, 0.01)	-0.07 (-0.17,0.03)	-0.11* (-0.20, -0.03)	0.01 (-0.06, 0.08)
State effects	NO	NO	NO	NO
Year effects	YES	YES	YES	YES
Adjusted R^2	0.11	0.08	0.12	0.06
Observations	816	816	816	816
F	26.33	17.74	28.57	14.9
DF	811	811	811	811
P	< 0.05	<0.05	<0.05	<0.05

Table 12. Spatial analysis model exploration for significant intervention effect

$$Y = \alpha + \beta_y + \beta_i + \beta_t + \gamma D_{it} + \varepsilon_{gt}$$

Model input	Raw NCDR	Raw NCDR	Smooth NCDR	Smooth NCDR
Outcome	Membership in a cluster	Membership in a cluster core	Membership in a cluster	Membership in a cluster core
Level 1: Aspatial Logistic Regression fit:	Poor	Poor	Poor	Poor
R^2	0.1062	0.0787	0.1182	0.0684
Global Moran's I	0.4017	0.4012	0.4671	0.5128
P-value	0.001*	0.001*	0.001*	0.001*
Level 2: GLMM deworming estimate	-0.72 (-1.67, 0.23)	-	-0.99* (-0.03, -1.94)	-
Global Moran's I	0.0066	0.0161	0.0016	0.0138
P-value	0.089	0.004*	0.3120	0.005*
Level 3: Spatial Regression	-		-	
Spatial Error Dependence:	-	Present	-	Present
Spatial Lag Dependence:	-	Present	-	Present

Figures

Figure Set 1 (Figures 1-4): Figures depicting the time trend of aspatial outcome variables throughout the entire study period.

Fig. 1. Leprosy new case detection rate over the entire study period, stratified by intervention and control group.

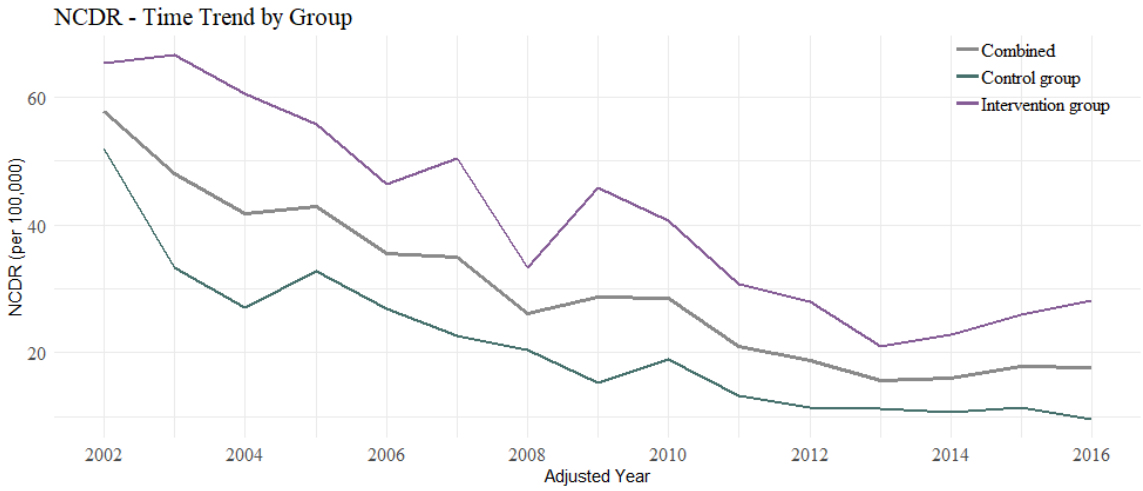


Fig. 2. % multibacillary cases over the entire study period, stratified by intervention and control group.

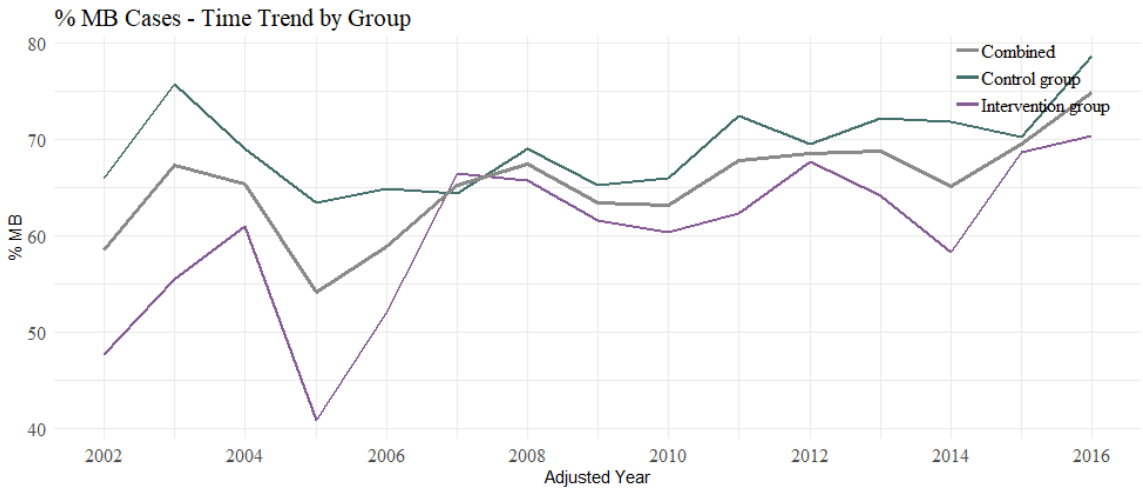


Fig. 3. Pediatric leprosy new case detection rate over the entire study period, stratified by intervention and control group.

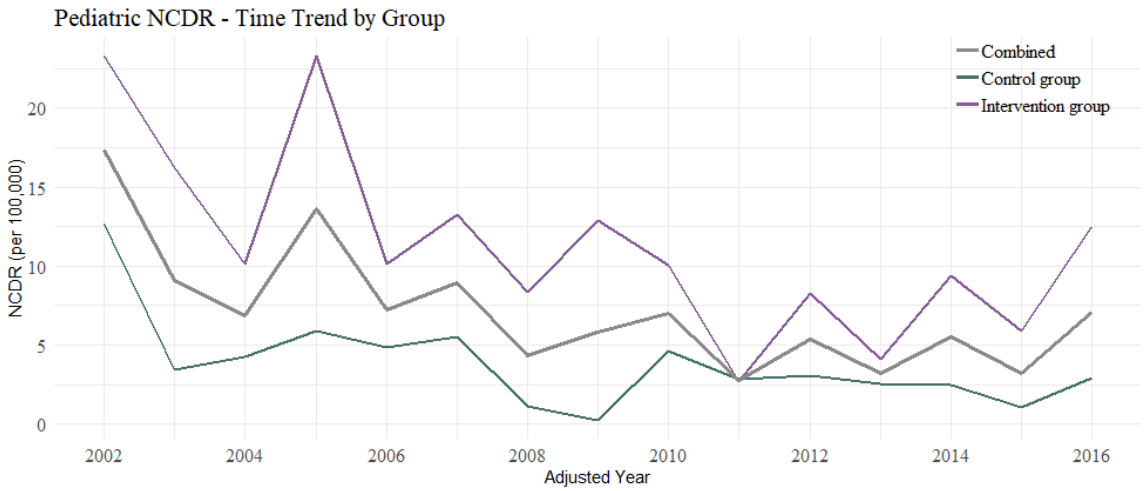


Fig. 4. Pediatric % multibacillary cases over the entire study period, stratified by intervention and control group.

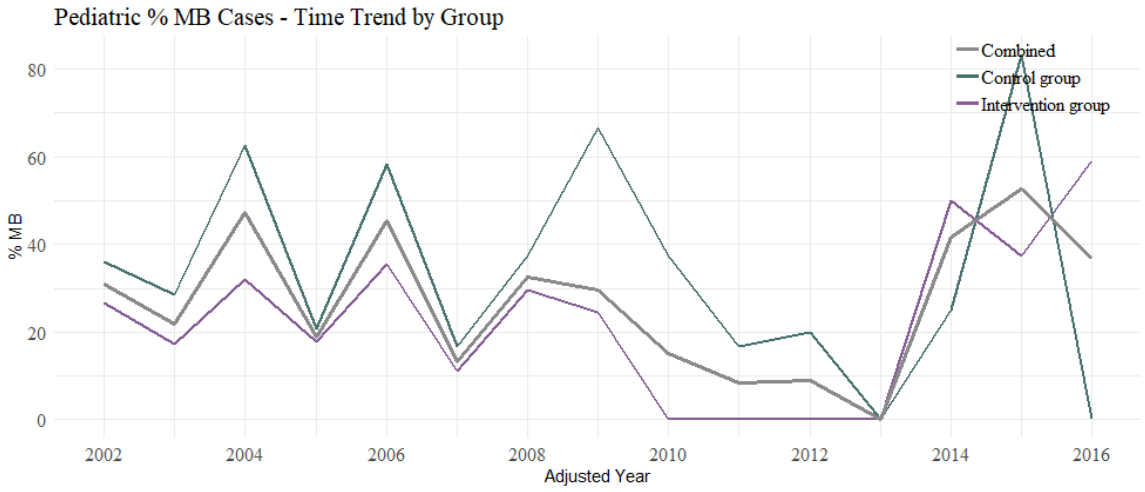


Figure Set 2 (Figures 5-8): Figures depicting the time trend of aspatial outcome variables in the pre-intervention study period. These figures were used to verify

whether the parallel trends assumption was met after the outcome variables were transformed for model fitting purposes.

Fig. 5. Leprosy new case detection rate (cube-root transformed) prior to introduction of intervention, stratified by intervention and control group. This figure was used to visually evaluate the parallel trends assumption for model 1.

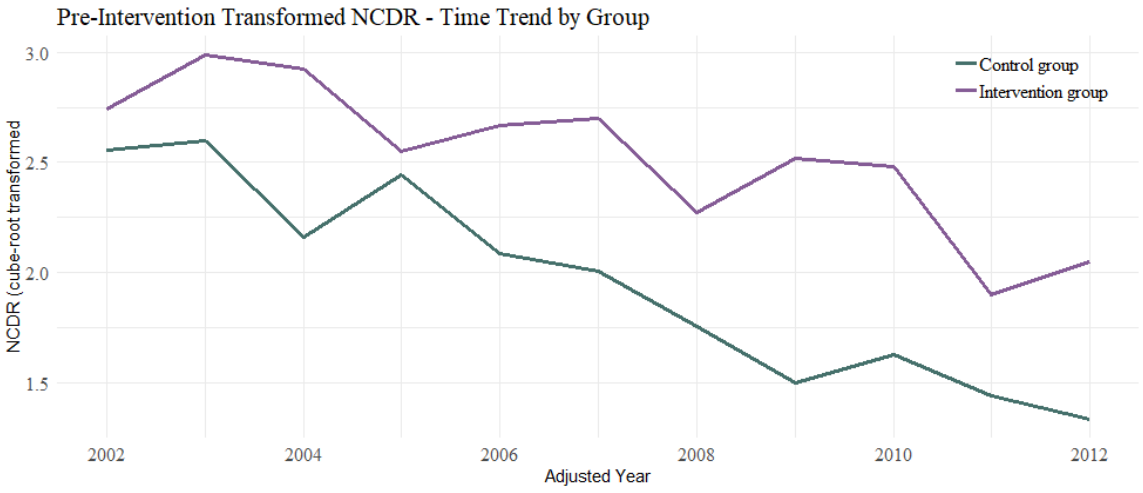


Fig. 6. Proportion of leprosy cases categorized as MB (cube-root transformed) prior to introduction of intervention, stratified by intervention and control group. This figure was used to visually evaluate the parallel trends assumption for model 2.

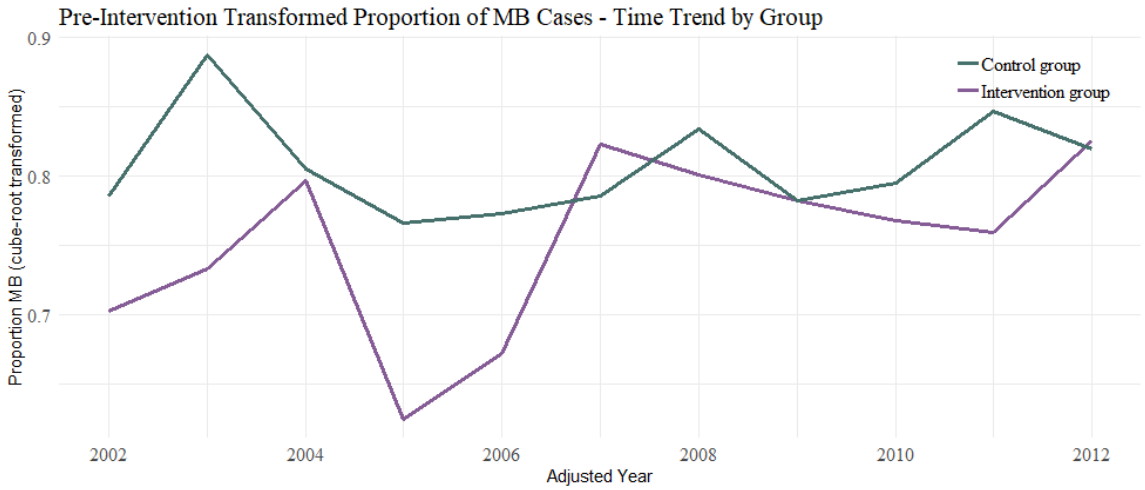


Fig. 7. Pediatric leprosy new case detection rate (cube-root transformed) prior to introduction of intervention, stratified by intervention and control group. This figure was used to visually evaluate the parallel trends assumption for model 3.

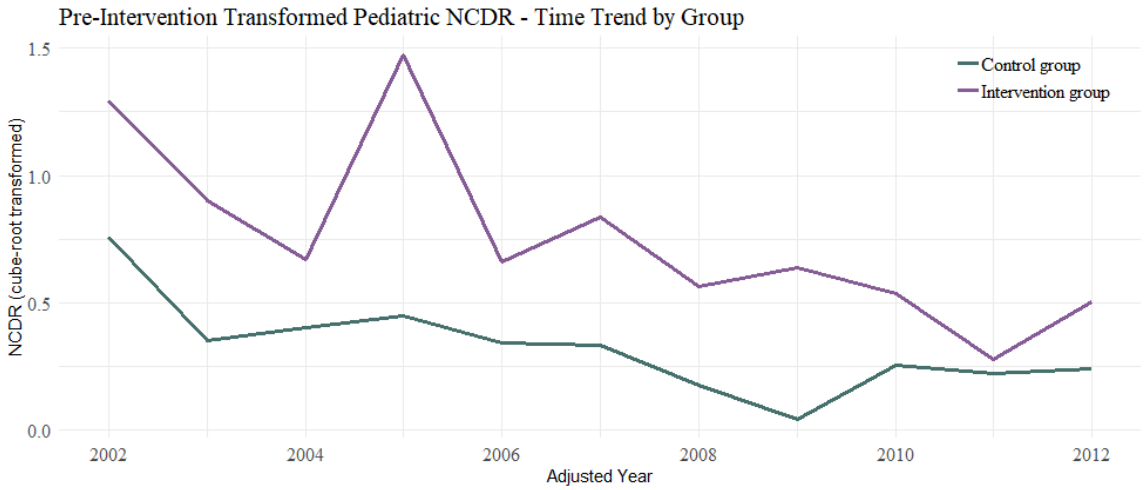


Fig. 8. Proportion of pediatric leprosy cases categorized as MB (cube-root transformed) prior to introduction of intervention, stratified by intervention and control group. This figure was used to visually evaluate the parallel trends assumption for model 4.

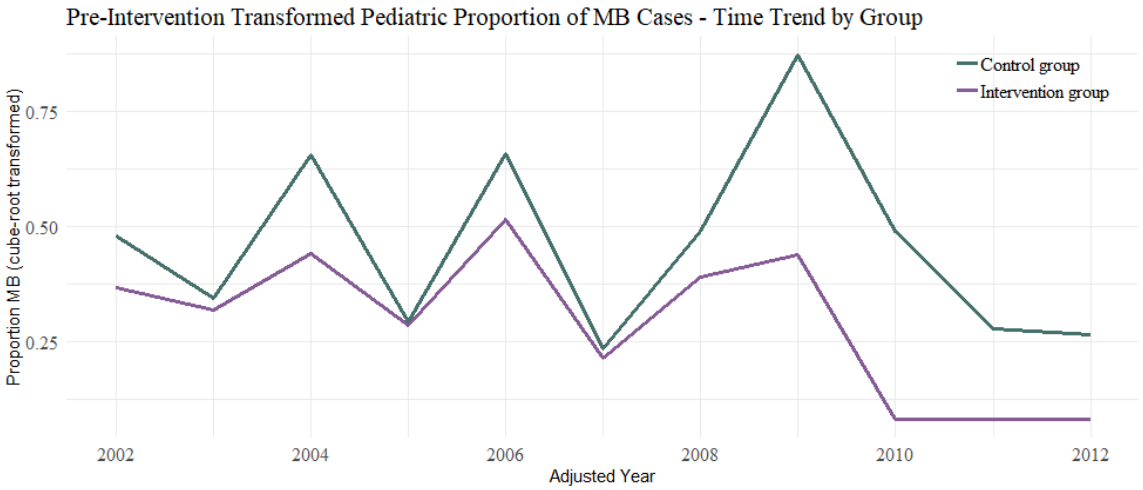


Figure set 3 (Figures 9-11, 16-17): Maps depicting the time trend of NCDR and hotspots identified by the Getis-Ord (G*) clustering test.

Fig. 9. Panel map of leprosy NCDR in Vale do Rio Doce, Minas Gerais, Brazil from 2009-2016.

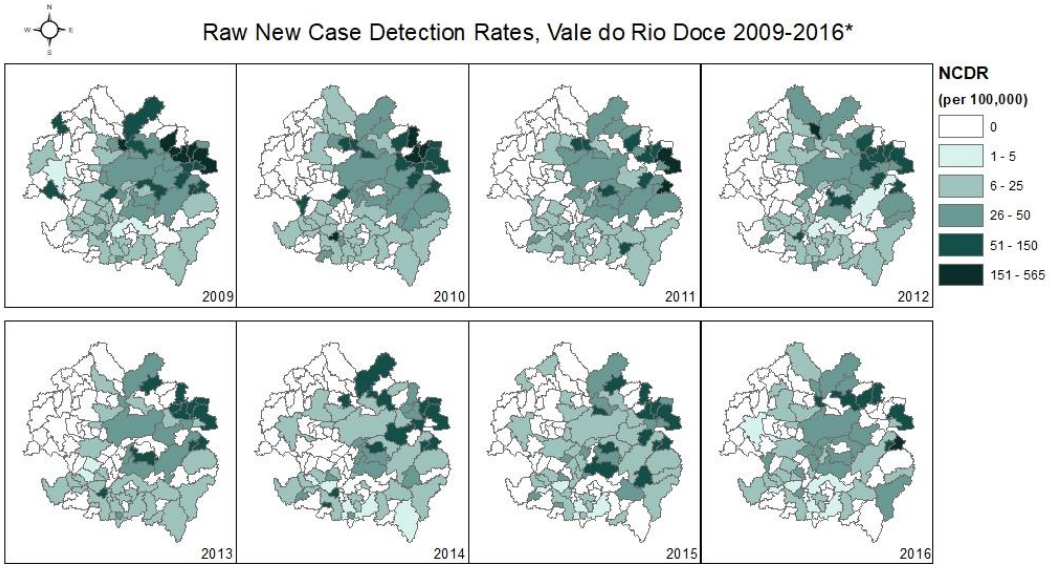


Fig. 10. Panel map of spatial empirical Bayesian smoothed leprosy NCDR in Vale do Rio Doce, Minas Gerais, Brazil from 2009-2016.

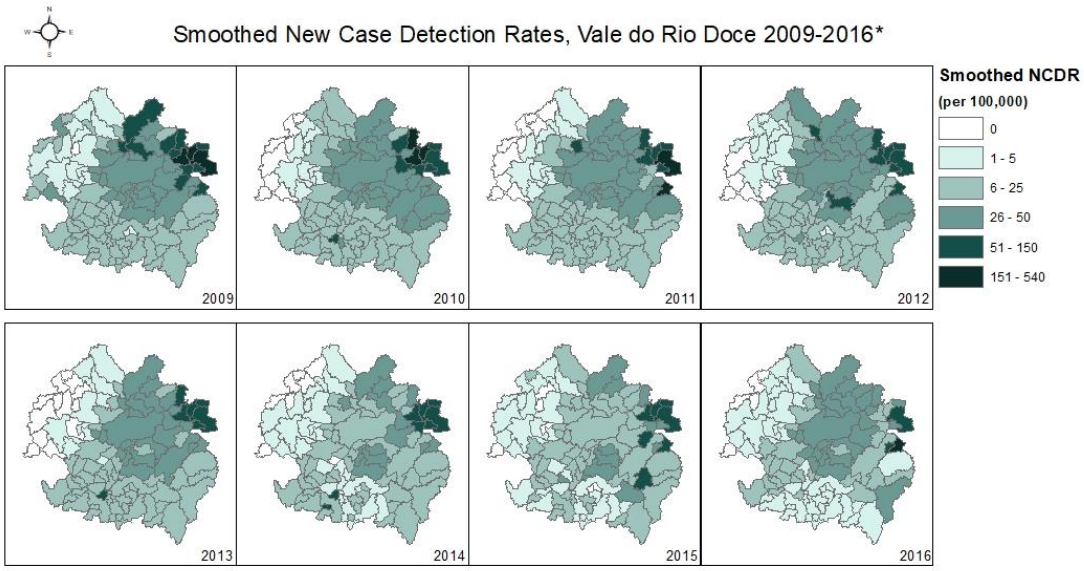


Fig. 11. Map identifying all municipalities selected to receive school-based deworming, Vale do Rio Doce, Minas Gerais, Brazil, at any time between 2013-2016.

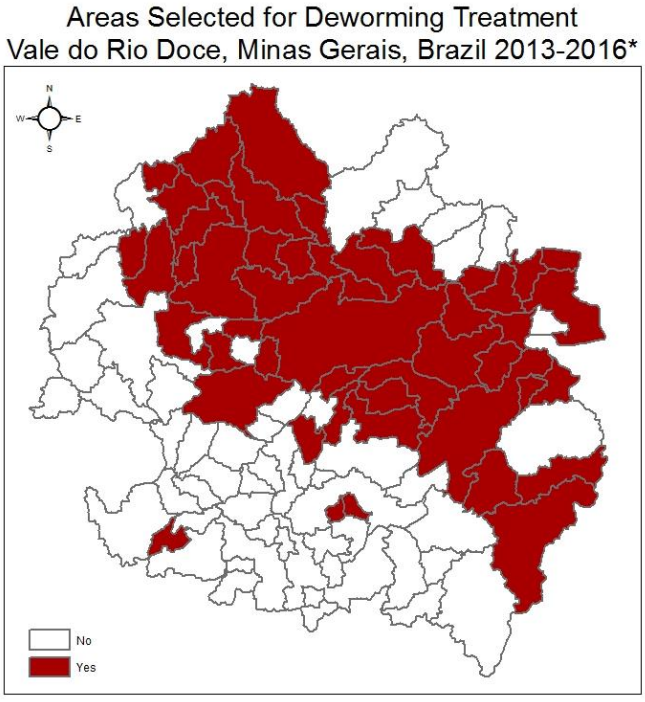


Figure Set 4 (Figures 12-15): Figures depicting the time trend of spatial outcome variables throughout the entire study period.

Fig. 12. Number of municipalities included in a hotspot cluster using raw rates over the entire study period, stratified by intervention and control group.

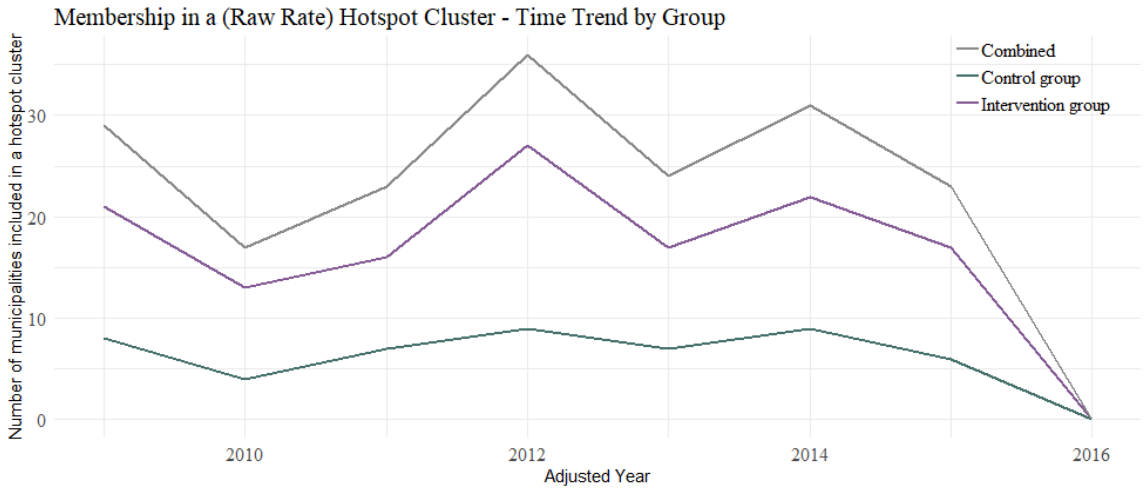


Fig. 13. Number of municipalities included in a hotspot cluster core using raw rates over the entire study period, stratified by intervention and control group.

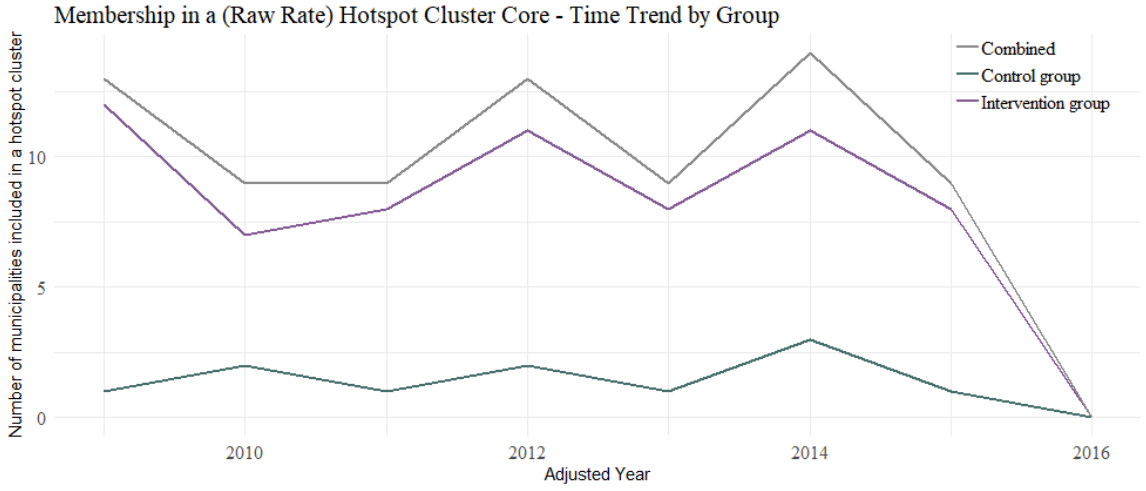


Fig. 14. Number of municipalities included in a hotspot cluster using smoothed rates over the entire study period, stratified by intervention and control group.

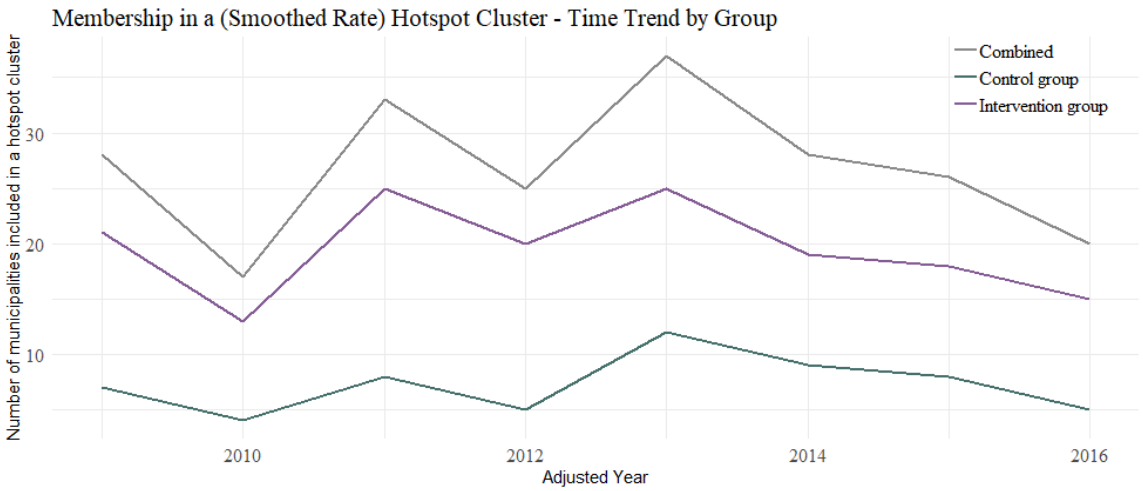


Fig. 15. Number of municipalities included in a hotspot cluster core using smoothed rates over the entire study period, stratified by intervention and control group.

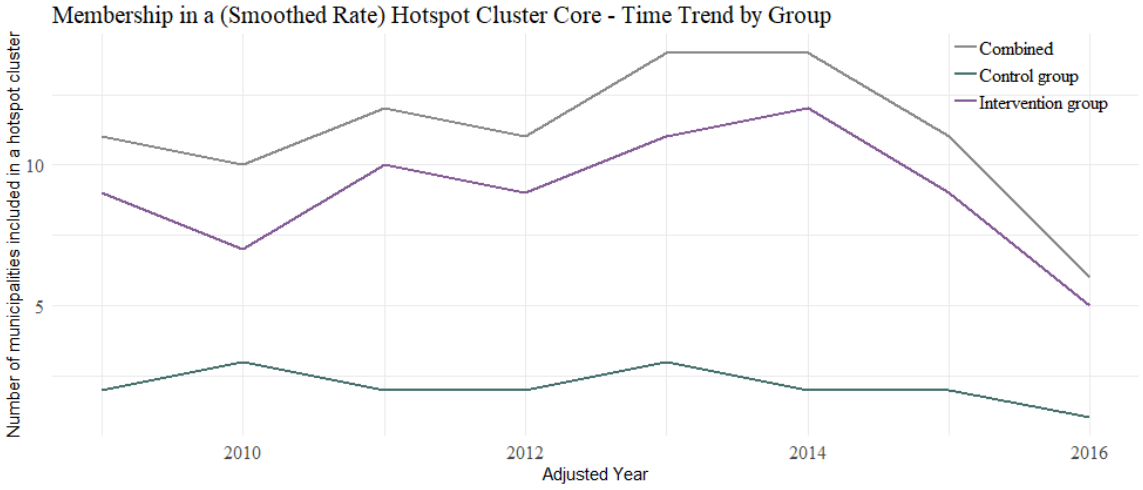


Figure set 3 cont.

Fig. 16. Panel map of leprosy NCDR hotspots in Vale do Rio Doce, Minas Gerais, Brazil from 2009-2016.

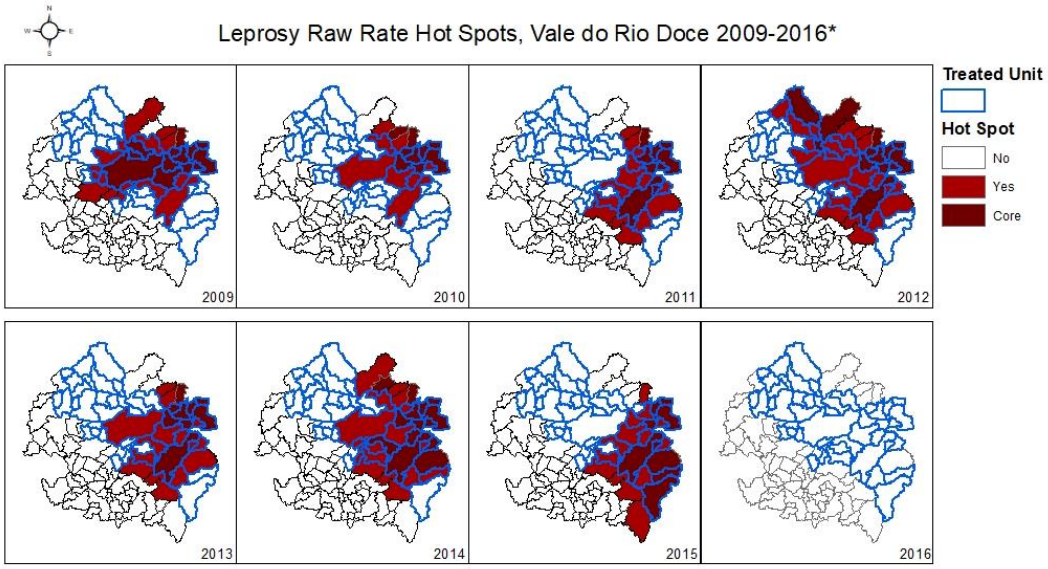


Fig. 17. Panel map of spatial empirical Bayesian smoothed leprosy hotspots in Vale do Rio Doce, Minas Gerais, Brazil from 2009-2016.

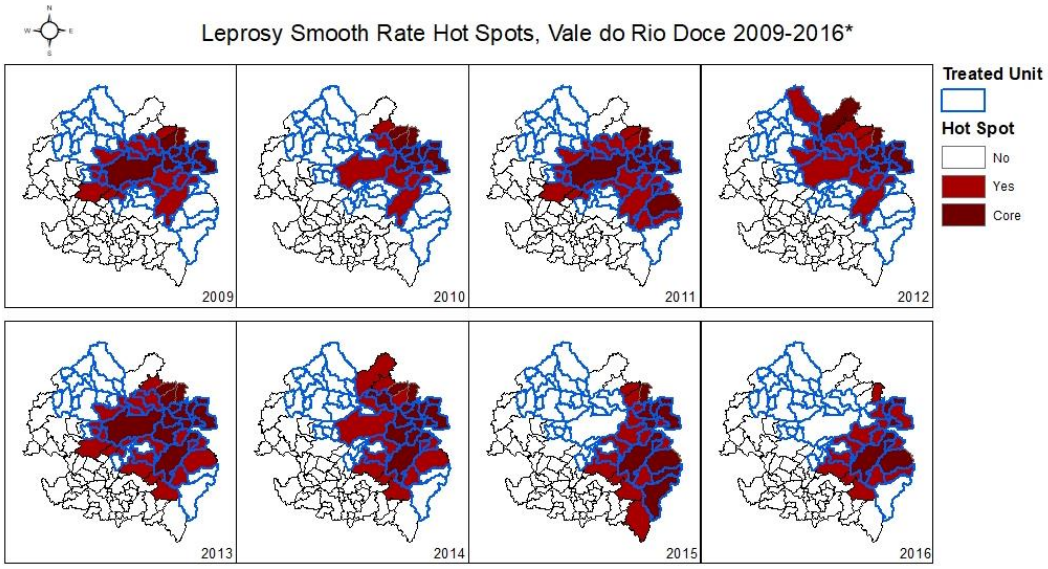


Figure Set 5 (Figures 18-21): Figures depicting the time trend of spatial outcome variables throughout the pre-intervention study period. These figures were used to verify whether the parallel trends assumption was met.

Fig. 18. Number of municipalities included in a hotspot cluster using raw rates prior to introduction of intervention, stratified by intervention and control group. This figure was used to visually evaluate the parallel trends assumption for the raw NCDR cluster model.

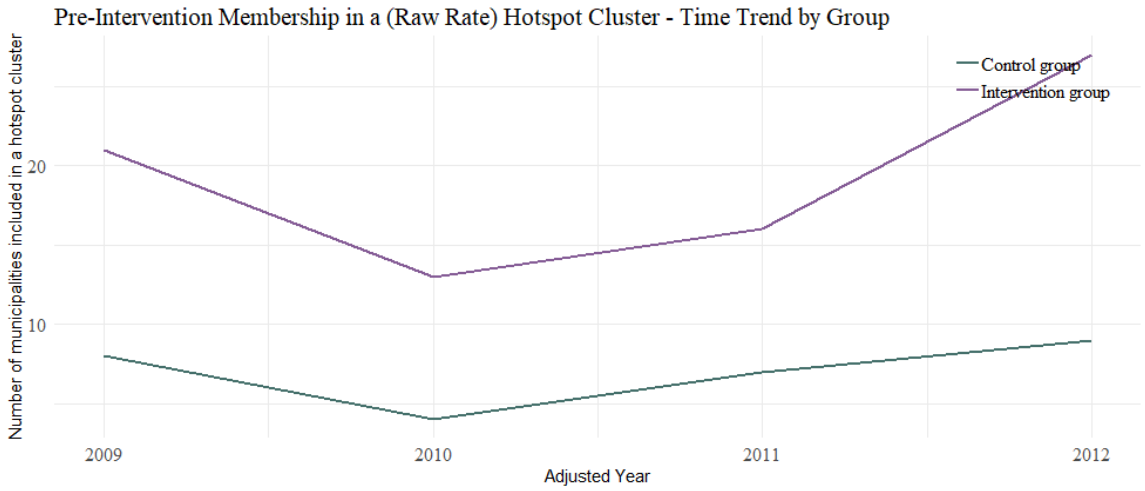


Fig. 19. Number of municipalities included in a hotspot cluster core using raw rates prior to introduction of intervention, stratified by intervention and control group. This figure was used to visually evaluate the parallel trends assumption for the raw NCDR cluster core

model.

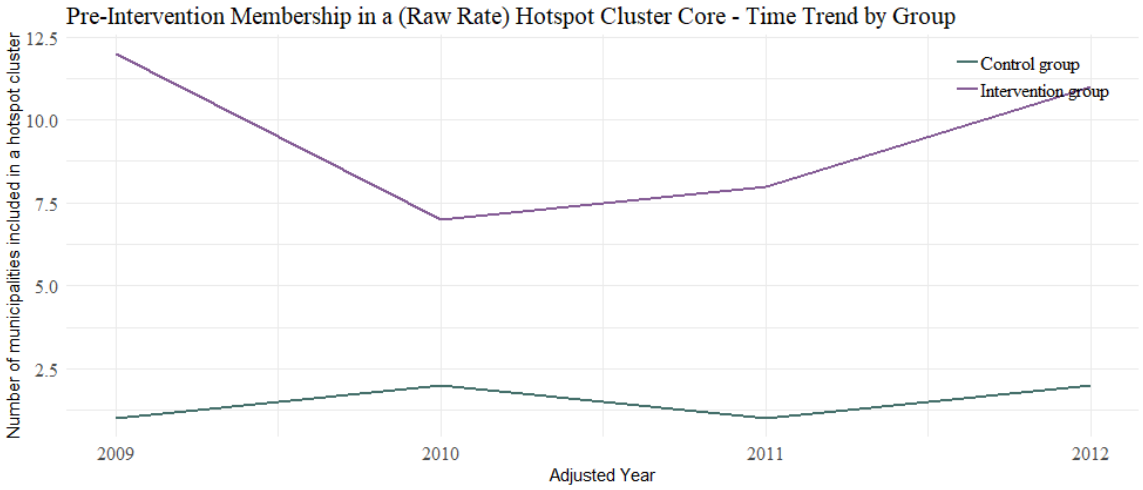


Fig. 20. Number of municipalities included in a hotspot cluster using smoothed rates prior to introduction of intervention, stratified by intervention and control group. This figure was used to visually evaluate the parallel trends assumption for the smoothed NCDR cluster model.

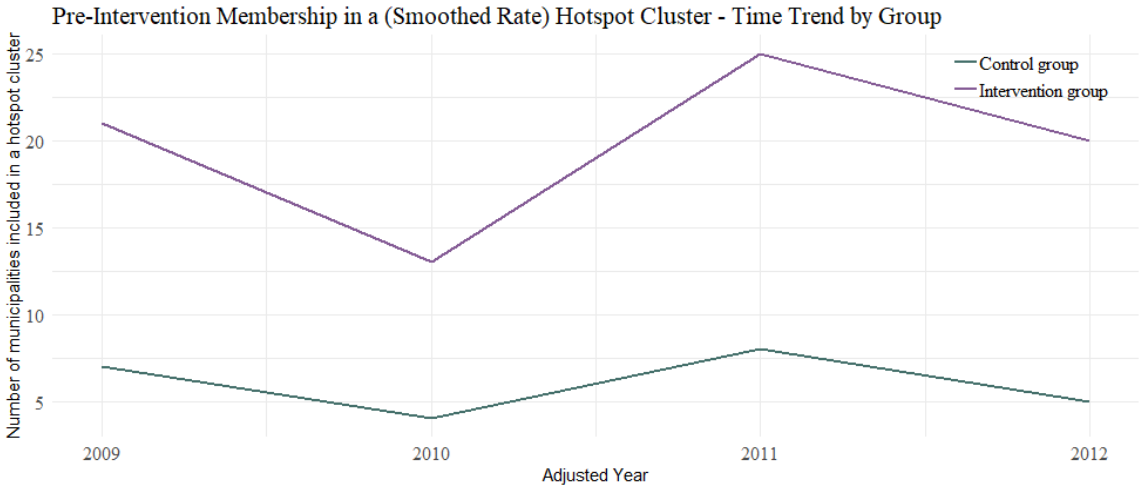
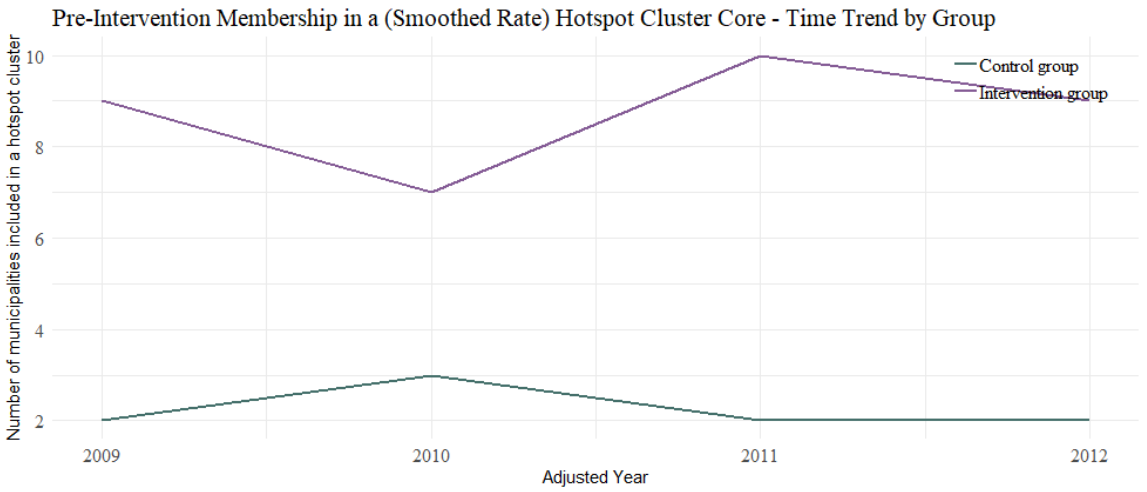


Fig. 21. Number of municipalities included in a hotspot cluster core using smoothed rates prior to introduction of intervention, stratified by intervention and control group. This

figure was used to visually evaluate the parallel trends assumption for the smoothed NCDR cluster core model.



Chapter III: Summary, Public Health Implications, Possible Future Directions

This study used a combination of spatial and aspatial methods to evaluate the observed impact of a school-based deworming program on leprosy new case detection rates and percent multibacillary cases among the total population and the pediatric subpopulation in the mesoregion of Vale Do Rio Doce, located within the state of Minas Gerais, Brazil. A difference-in-differences analysis was used to determine the effect of treatment in the presence of municipality-specific and time-specific trends in the data, and a cluster analysis, paired with several model types, was undertaken to consider the spatial structure of case clustering. While results of the models largely indicated a negative, non-significant effect of the intervention on leprosy outcomes, interesting trends in leprosy outcomes were observed, indicating the need for more research to determine whether anthelmintic treatment has an effect on leprosy incidence and its polarization to the multibacillary subtype. A stronger characterization of this relationship is necessary to understand the potential impacts of disease control of one disease on the distribution of the other, as well as to highlight opportunities for integrated disease control, as the Brazil Ministry of Health was able to do in the implementation of its plan. Both considered neglected tropical diseases, STH infection control and leprosy elimination programs operate with limited financial resources and often a shortage of research to support decision-making at the operational level. Combined studies could highlight opportunities for joint disease control and elimination. This study supports the combined use of spatial and aspatial methods as a model to develop deeper understanding of disease co-infection and its causes and effects.

The incorporation of tools such as GIS into epidemiological studies offers a useful strategy to capture the spatial component of disease distribution within epidemiologic estimates.

Appendices

Figure Set 6 (Figures 22-25): Figures depicting the time trend of outcome variables throughout the pre-intervention study period. These figures were used to verify whether the parallel trends assumption was met prior to variable transformation for the purpose of model fitting.

Fig. 22. Leprosy new case detection rate (untransformed) prior to introduction of intervention, stratified by intervention and control group.

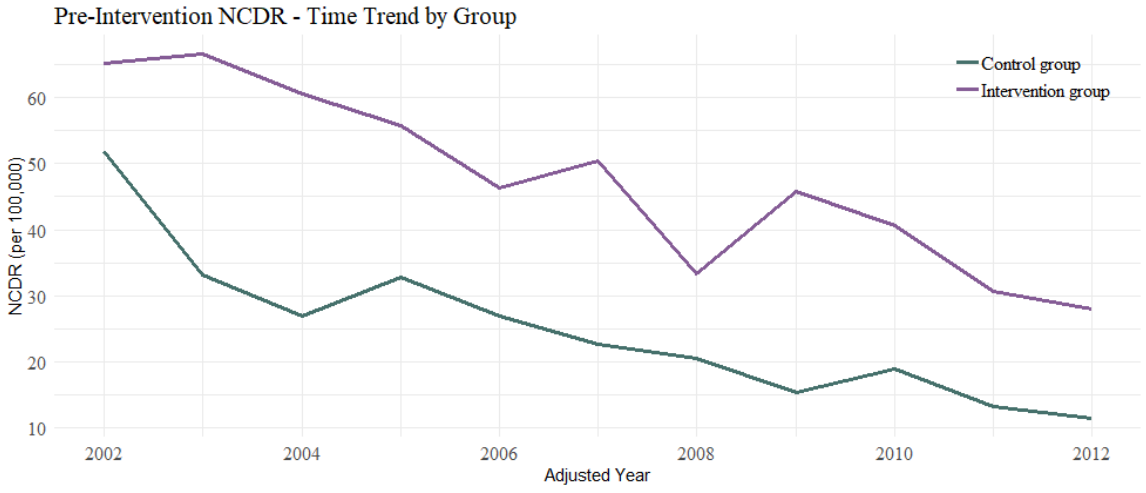


Fig. 23. Proportion of leprosy cases categorized as MB (untransformed) prior to introduction of intervention, stratified by intervention and control group.

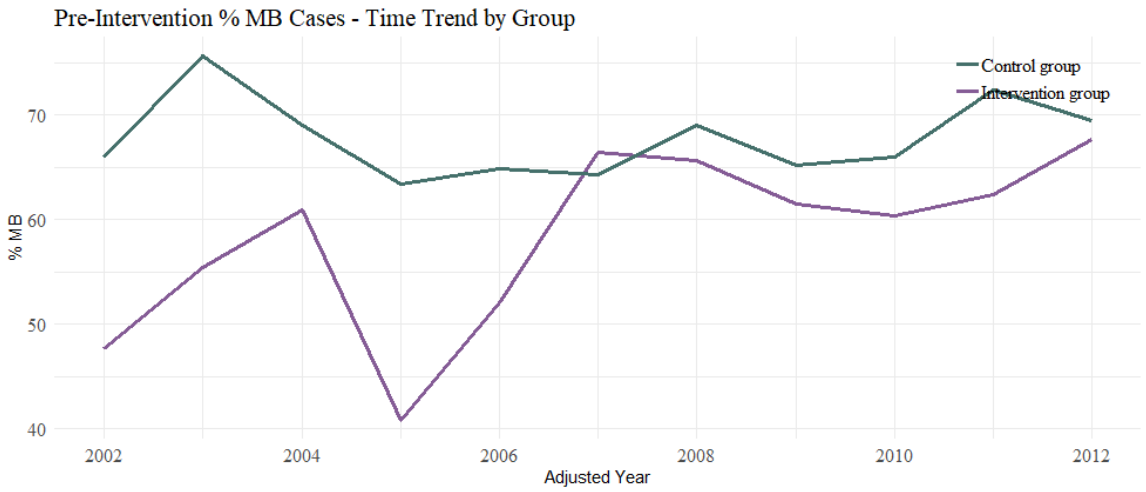


Fig. 24. Pediatric leprosy new case detection rate (untransformed) prior to introduction of intervention, stratified by intervention and control group

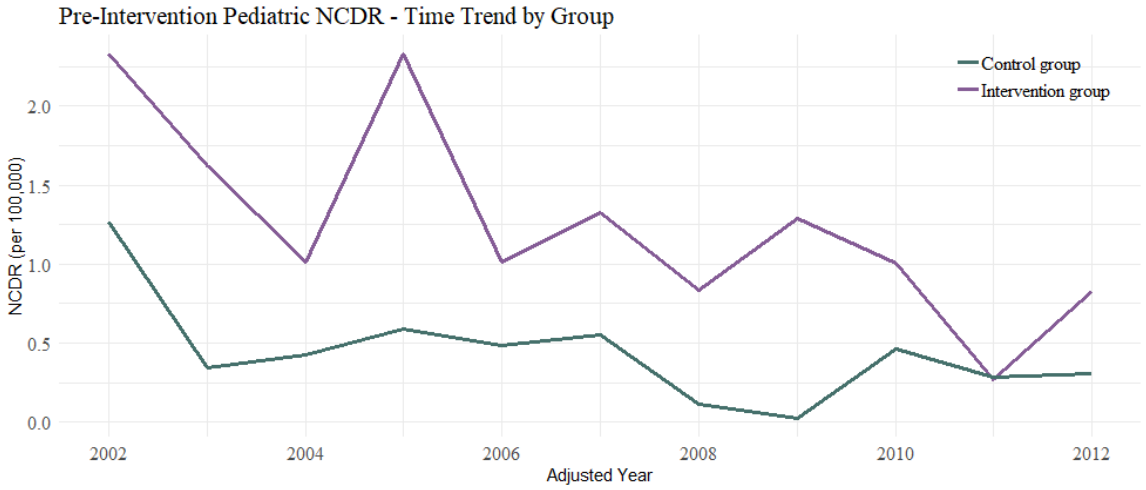


Fig. 25. Proportion of pediatric leprosy cases categorized as MB (untransformed) prior to introduction of intervention, stratified by intervention and control group

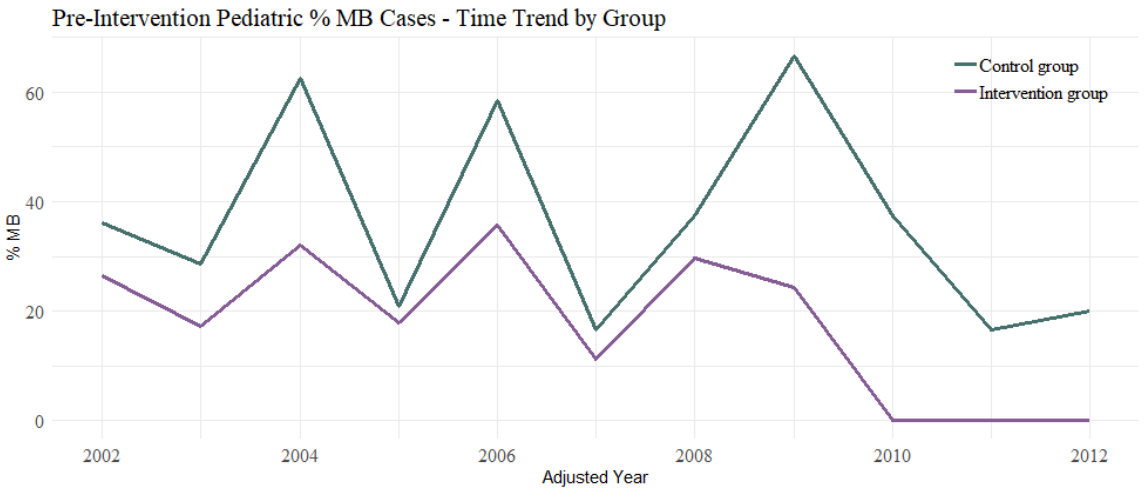


Figure set 7: Maps providing additional information on the study area, pediatric new case detection rate, and pediatric and total percent multibacillary cases.

Fig. 26. Panel map of % MB cases in Vale do Rio Doce, Minas Gerais, Brazil from 2009-2016.

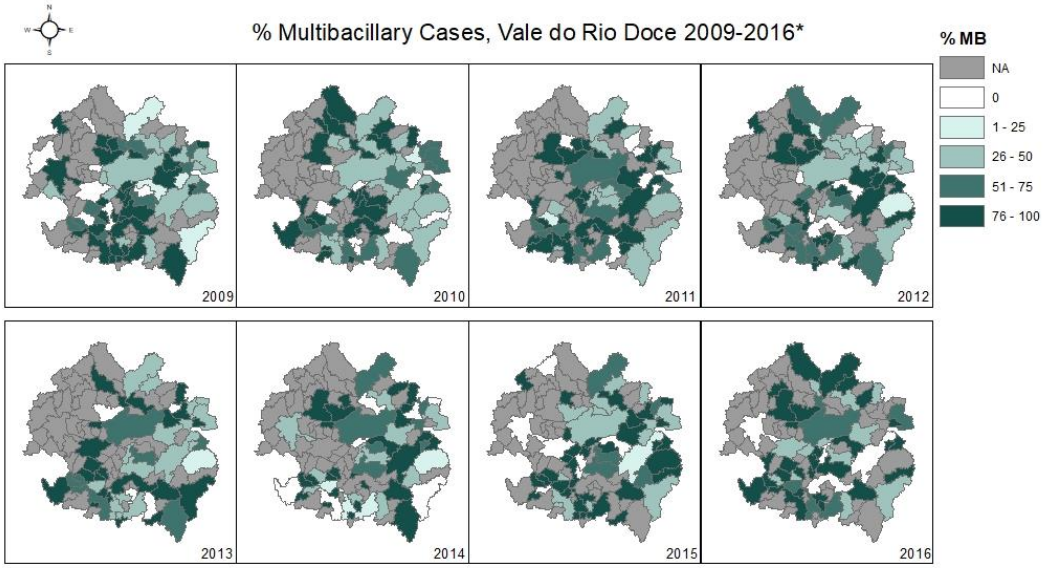


Fig. 27. Panel map of pediatric leprosy new case detection rate in Vale do Rio Doce, Minas Gerais, Brazil from 2009-2016.

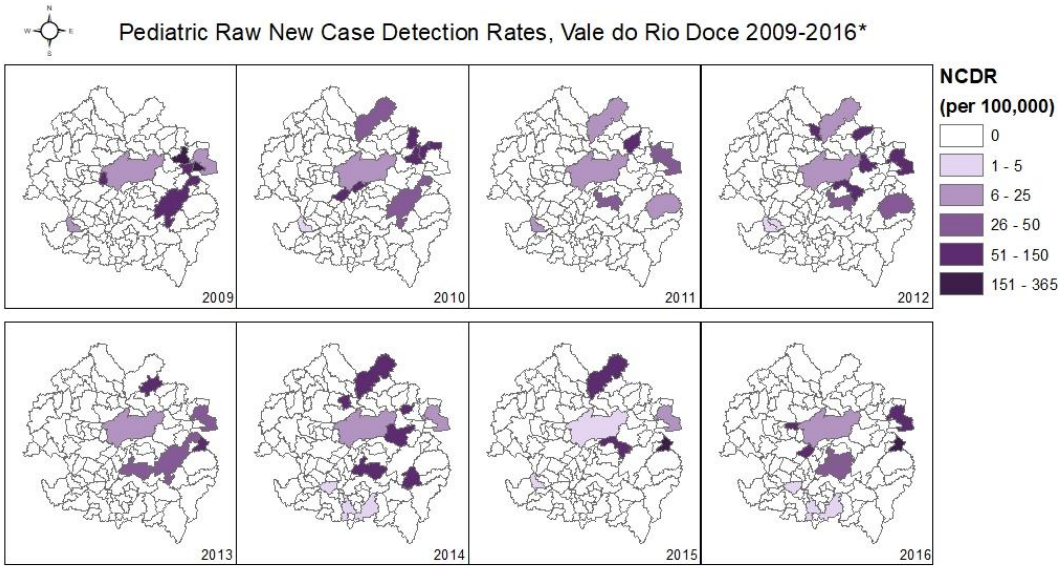
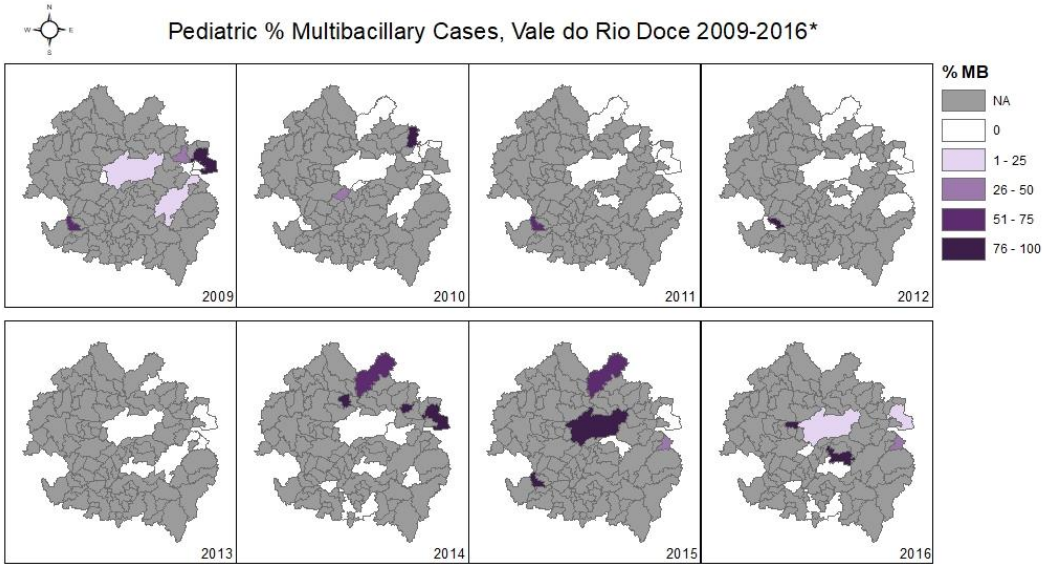


Fig. 28. Panel map of pediatric leprosy % MB cases in Vale do Rio Doce, Minas Gerais, Brazil from 2009-2016.



Appendix Table 1: Variable definitions corresponding to Table 2. Variables were extracted from a 2010 dataset, reflected in the definitions of cutpoints such as poverty level.

Fertility rate	Average number of children a woman has during reproductive years (15-49).
HH density	Ratio of the population living in households with a density greater than 2 to the total population multiplied by 100. Household density is given by the ratio of the total number of household members to the total number of bedrooms.
Child poverty	Proportion of children (under age 14) living in a household with monthly per capita income equal to or less than R$140.
Poverty	Proportion of individuals living in a household with monthly per capita income equal to or less than R$140.
Child indigence	Proportion of children (under age 14) living in a household with monthly per capita income equal to or less than R$70.
Indigence	Proportion of individuals living in a household with monthly per capita income equal to or less than R$70.
Municipal Human Development Index	Geometric mean of Income, Education and Longevity human development indices, with equal weights.
Inadequate access to clean water and proper sewage	Ratio of individuals living in households whose water is not supplied by a general network and whose sewerage is not covered by a sewage collection system or septic tank to the total population multiplied by 100.
Under 1 mortality	Number of deaths per 1,000 births in the first year of life.
Under 5 mortality	Number of deaths per 1,000 births within the first five years of life.
Life expectancy	Average life expectancy at birth.
GINI (income distribution)	Inequality measure based on per capita income distribution. Range: 0 (no inequality) – 1 (complete inequality).
Median income	Average earnings of all employed individuals over age 18.
Individuals vulnerable to poverty that travel > 1 hour to work	Ratio of people living in households vulnerable to poverty (per capita income less than 1/2 minimum wage) and who travel over one hour to work to total employed persons multiplied by 100.
*Variables included only individuals living in permanent, private households **Data retrieved from Atlas of Human Development in Brazil	

Appendix Table 2: R packages used for analysis

AmostraBrasil	Celso Stephan;Ricardo Cordeiro (2016). AmostraBrasil: Generates Samples or Complete List of Brazilian IBGE (Instituto Brasileiro De Geografia e Estatistica) Census Households, Geocoding it by Google Maps. R package version 1.2. https://CRAN.R-project.org/package=AmostraBrasil
stats	R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

sandwich	Zeileis A (2004). "Econometric Computing with HC and HAC Covariance Matrix Estimators." _Journal of Statistical Software_, *11*(10), 1-17. doi: 10.18637/jss.v011.i10 (URL: http://doi.org/10.18637/jss.v011.i10). Zeileis A (2006). "Object-Oriented Computation of Sandwich Estimators." _Journal of Statistical Software_, *16*(9), 1-16. doi: 10.18637/jss.v016.i09 (URL: http://doi.org/10.18637/jss.v016.i09). Berger S, Graham N, Zeileis A (2017). "Various Versatile Variances: An Object-Oriented Implementation of Clustered Covariances in R." Technical Report 2017-12, Working Papers in Economics and Statistics, Research Platform Empirical and Experimental Economics, Universität Innsbruck. <URL: http://EconPapers.RePEc.org/RePEc:inn:wpaper:2017-12>.
lmtest	Achim Zeileis, Torsten Hothorn (2002). Diagnostic Checking in Regression Relationships. R News 2(3), 7-10. URL https://CRAN.R-project.org/doc/Rnews/
betareg	Francisco Cribari-Neto, Achim Zeileis (2010). Beta Regression in R. Journal of Statistical Software 34(2), 1-24. URL http://www.jstatsoft.org/v34/i02/.