

Epidemiology of yaws in the Solomon Islands and the impact of a trachoma control programme



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Declaration:

I declare that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated within the thesis.

A handwritten signature in black ink, appearing to be 'MM', with a large, sweeping flourish extending to the right.

Signature Date: 30th May 2016

Michael Marks

Preface

This thesis is presented as a 'Research Paper Style Thesis' in accordance with submission guidance provided by the London School of Hygiene and Tropical Medicine. Six of the chapters comprise a total of eight papers that have been published, submitted for publication or that are in preparation for submission to particular peer-reviewed journals. These are highlighted in italics in the Table of Contents. In view of the differing requirements of the journals in which the work has been published there is by necessity some repetition of material and variation in the formatting of these chapters. Publication details and acknowledgement of co- author contributions are included on the individual cover sheets for each paper. The remainder of the thesis is comprised of 'linking material' and includes an introduction to the overall research project.

All material within this thesis was written by Michael Marks.

Abstract

Yaws is a re-emerging endemic treponemal infection. The Pacific Islands are believed to be a major focus of yaws worldwide. WHO has recently developed a strategy for global yaws eradication based predominantly on community mass treatment with azithromycin at a dose of 30mg/kg (max 2g). Mass treatment with azithromycin is also key to the WHO strategy for trachoma elimination, although the dose used is lower (20mg/kg – max 1g). In areas where trachoma and yaws are co-endemic, mass treatment of populations as part of trachoma control programmes might aid yaws eradication efforts, but could also have negative consequences if drug resistance were to be encouraged.

Prior to mass treatment with azithromycin, the prevalence of clinical and serological evidence of yaws in the Solomon Islands was found to be high. Household contact with a seropositive individual was a strong risk factor for infection, especially if the contact also had an active skin lesion. Village level seroprevalence was shown to be the strongest risk factor for infection. *Haemophilus ducreyi* was identified as the likely cause of a large proportion of ulcerative skin lesions amongst children, which were clinically indistinguishable from those of yaws.

A single round of mass treatment with azithromycin at a dose of 20mg/kg significantly decreased the prevalence of both clinical signs of yaws and serological evidence of active infection. This effect was shown to extend to at least 18 months after mass treatment in the absence of any further intervention. Not receiving treatment with azithromycin was the major risk factor for seropositivity following MDA at both 6 and 18 months of follow-up.

A rapid diagnostic test for syphilis was shown to also have potential value for use in yaws. The sensitivity of the test was strongly associated with the antibody titre on gold standard testing, suggesting the test may be most appropriate for testing individuals with suspected active yaws where antibody titres are higher.

Mathematical modelling data were used to establish the minimum number of rounds and coverage that are likely to be required to interrupt transmission. Consistent with the findings of the post-MDA prevalence surveys, the model predicted that high coverage – ideally above 80% - is likely to be required to interrupt transmission.

This PhD has addressed several key questions about the epidemiology of yaws. Even within endemic populations, the disease is highly focal. Integration of rapid diagnostic tests into routine surveillance may help improve data quality and guide yaws elimination efforts at a national level. Given the strong association between coverage of mass treatment and risk of infection, new strategies to increase the reach of yaws eradication strategies are needed. Mathematical modelling may be of use in informing the design of these interventions.

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I am enormously grateful to many people for their support and assistance over the course of my PhD. I was incredibly lucky to be supervised by David Mabey and Anthony Solomon, who set me on the road to the Solomon Islands and have provided inspiration, intellectual guidance, and additional commas in equal measure. It was a privilege to undertake my PhD with them both.

The LSHTM/Wellcome Trust Fellowship in International Health has given me wonderful support and opportunities over the course of my PhD and I have benefitted hugely from the insight of the other Fellows on the scheme. I hope that the skills, opportunities and relationships developed over my PhD will set me on the road to an independent academic career. I would particularly like to thank Eleanor Martins and Tamara Hurst for their help with administering my grant through LSHTM.

I have benefitted hugely from the insight and experience of many people at LSHTM. Martin Holland, Robin Bailey, Simon Brooker and Alison Grant helped shape my project in its early stage and ensured that my PhD was both academically focused and provided the training and skills I needed. Christian Bottomley has provided valuable insight in to statistical issues throughout the PhD, whilst Sebastian Funk helped steer me through my introduction to mathematical modelling approaches to epidemiological questions.

I was lucky enough to spend time at the Centers for Disease Control and Prevention during my Fellowship and I am enormously grateful to Cheng Chen and Allan Pillay for hosting me in their team in Atlanta, and to Kai-Hua Chi for training me on the use of the real-time PCR assays used in this project. I would also like to thank Diana Martin, who provided guidance on the collection of dried blood spot samples. I have also had extremely fruitful conversations and discussions with many other colleagues, in particular Georgina Kilua, Zaixing Zhang, Seyha Ros, Kingsley Asiedu and Oriol Mitjà.

The fieldwork for this study would not have been possible without the support and contributions of my colleagues in the Solomon Islands, whose insights in to the Pacific and enthusiasm for my work were invaluable. Audrey Aumua made me feel incredibly welcome in the WHO office in the Solomon Islands and provided a wonderful base in Honiara. Willie Horoto at the National Medical Stores and Elliot Puiahi at the National Referral Hospital made the logistics of working in challenging field conditions much easier. Most importantly Oliver Sokana was an incredible colleague and friend, and helped me get more out of my time in the Solomon Islands than I could have hoped for.

I would never have started down the road to tropical medicine without the advice and support of Philip Gothard, Mahdad Noursadeghi, and most importantly Tom Doherty, who was an inspirational teacher and mentor. I am grateful to them all for their support early in my clinical-academic career.

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Acronyms

CDC	Centers for Disease Control and Prevention
GPS	Global Positioning System
LSHTM	London School of Hygiene & Tropical Medicine
MDA	Mass Drug Administration
MHMS	Ministry of Health and Medical Services
NHP	Non-Human Primates
NTD	Neglected Tropical Diseases
ODK	Open Data Kit
PCR	Polymerase Chain Reaction
RDT	Rapid Diagnostic Test
rRNA	Ribosomal Ribose Nucleic Acid
RT-PCR	Real-Time Polymerase Chain Reaction
RPR	Rapid Plasma Reagin
SAFE	Surgery, Antibiotics, Facial Cleanliness, Environmental Improvement
TCT	Total Community Treatment
TF	Trachomatous Inflammation - Follicular
TPHA	Treponema Pallidum Haem-Agglutination
TPPA	Treponema Pallidum Particle Agglutination
TTT	Total Targeted Treatment
VDRL	Venereal Disease Research Laboratory
WASH	Water, Sanitation and Hygiene
WHO	World Health Organization

List of contributors to the research presented in this thesis

Name	Position/Institution	Contribution
David Mabey	Professor of Communicable Disease, CRD, ITD, LSHTM	PhD Supervisor
Anthony Solomon	Honorary Senior Lecturer, CRD, ITD, LSHTM	PhD Co-Supervisor
Christian Bottomley	Lecturer in Medical Statistics, IDE, EPH, LSHTM	Member of advisory committee. Statistical support for risk factor analysis
Sebastian Funk	Lecturer in Mathematical Modelling, IDE, EPH, LSHTM	Member of advisory committee. Support for mathematical modelling study.
Martin Holland	Reader of Microbial Immunity, CRD, ITD, LSHTM	Member of upgrading committee
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Alison Grant	Professor of International Health	Member of advisory committee
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Willie Horoto	Head of National Medical Stores, Solomon Islands Ministry of Health and Medical Services	Essential logistic support for fieldwork
Oliver Sokana	Focal Person for Trachoma, Solomon Islands Ministry of Health and Medical Services	Essential logistic support for fieldwork
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Ventis Vahi	Deputy Director, National Health Training and Research Institute, Solomon Islands Ministry of Health and Medical Services	Essential logistic support for fieldwork
Allan Pillay	Research Microbiologist, Centres for Disease Control and Prevention	Support for performing RT-PCR on lesion samples
Cheng Chen	Microbiologist, Centres for Disease Control and Prevention	Support for performing RT-PCR on lesion samples
Oriol Mitjà	Associate Professor, ISGlobal	Input in to mathematical modelling study
Kingsley Asiedu	Medical Officer, World Health Organization	Input in to mathematical modelling study
Christopher Fitzpatrick	Economist, World Health Organization	Input in to mathematical modelling study

Thesis outline

The thesis consists of a mixture of explanatory material, data chapters based on published and submitted manuscripts and relevant linking material. Chapters highlighted in italics consist of papers that have been published or submitted for publication. The other chapters are linking material prepared for this thesis.

Chapter 1 is a review of the epidemiology, clinical features and management of yaws.

Chapter 2 provides an overview of the components of the PhD project and the scientific rationale for the study.

Chapter 3 addresses the first two objectives of my PhD and presents data on the pre-MDA epidemiology of yaws in the Solomon Islands

Chapter 4 addresses the third and fourth objectives of my PhD and includes two papers presenting data on the impact of community mass treatment with azithromycin on yaws.

Chapter 5 presents data on the validation of a rapid diagnostic serological test for yaws, addressing the fifth objective of my PhD.

Chapter 6 presents a mathematical modelling study undertaken to explore the likely programmatic requirements of a yaws eradication campaign, addressing the final objective of my PhD.

Chapter 7 provides an overall summary of the PhD in relation to my objectives.

Chapter 8 provides a commentary on the progress towards yaws eradication that has been made over the course of my PhD

Chapter 9 provides an overview of future research questions raised by the PhD.

Chapter 10 contains relevant appendices from the studies conducted during the PhD.

Chapter 1: Review of yaws epidemiology, clinical features and management



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RESEARCH PAPER COVER SHEET

PLEASE NOTE THAT A COVER SHEET MUST BE COMPLETED FOR EACH RESEARCH PAPER INCLUDED IN A THESIS.

SECTION A – Student Details

Student	MICHAEL MARKS
Principal Supervisor	DAVID MASEY
Thesis Title	EPIDEMIOLOGY OF YAWS IN THE SOLOMON ISLANDS AND THE IMPACT OF A TRACHOMA CONTROL PROGRAMME

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?	BRITISH MEDICAL BULLETIN		
When was the work published?	2015		
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Yaws

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Abstract

Introduction: Yaws, caused by *Treponema pallidum* ssp. *pertenue*, is endemic in parts of West Africa, Southeast Asia and the Pacific. The WHO has launched a campaign based on mass treatment with azithromycin, to eradicate yaws by 2020.

Sources of data: We reviewed published data, surveillance data and data presented at yaws eradication meetings.

Areas of agreement: Azithromycin is now the preferred agent for treating yaws. Point-of-care tests have demonstrated their value in yaws.

Areas of controversy: There is limited data from 76 countries, which previously reported yaws. Different doses of azithromycin are used in community mass treatment for yaws and trachoma.

Growing points: Yaws eradication appears an achievable goal. The programme will require considerable support from partners across health and development sectors.

Areas timely for developing research: Studies to complete baseline mapping, integrate diagnostic tests into surveillance and assess the impact of community mass treatment with azithromycin are ongoing.

Key words: yaws, syphilis, eradication, neglected tropical diseases

Introduction

Yaws is an infectious disease caused by *Treponema pallidum* ssp. *pertenue* and is one of the four treponemal diseases affecting humans.^{1,2} Unlike syphilis, which is caused by the almost identical *T. pallidum pallidum*, it is not sexually transmitted, but is thought to be spread by skin to skin contact in warm humid environments, and mother to child transmission is not seen. As with other treponemal infections, yaws causes primary, secondary and tertiary lesions, which predominantly affect the skin, bones and cartilage. Yaws was the first disease to be targeted for eradication by the World Health Organization (WHO), and mass screening and treatment programmes led by WHO reduced the global prevalence by >95% between 1950 and 1964, but it has re-emerged as an important public health problem in West Africa, Southeast Asia and the Pacific in recent years.³ The recent demonstration that a single oral dose of azithromycin is as effective as injectable penicillin in the treatment of yaws⁴ has prompted renewed interest in the possibility of yaws eradication, but barriers such as funding and access to azithromycin have to be overcome before this goal can be realized.

Epidemiology

Yaws is found in warm and humid environments⁵ and affects mostly children between 2 and 15 years old, who are considered as the reservoir for infections. The disease is spread by direct skin-to-skin, non-sexual, contact often after a cut or abrasion in the lower legs.⁶ There have been suggestions that flies may act as a vector for yaws,⁶ but there is no definitive proof that this occurs. Treponemal infections closely related to yaws and syphilis have been identified in primates, but there is no evidence to suggest that zoonotic transmission between humans and non-human primates occurs.⁷ Children born to mothers affected with yaws are generally unaffected, and most evidence seems to indicate that the disease is not acquired congenitally.

The early lesions of yaws are most infectious. It is estimated that patients are infectious for up to 12–18

months following primary infection,⁶ but relapsing disease can extend this period. The destructive lesions of late yaws are not infectious. In studies in both Papua New Guinea and the Solomon Islands, endemicity at the village level has been identified as the major risk factor for infection and re-infection following treatment.^{8,9} The disease primarily affects rural communities with low standards of hygiene, with incidence declining as social and economic status rise.

In the mid-20th century, yaws was reported to affect ~50 million individuals and to be endemic in at least 90 countries¹⁰ in South America, the Caribbean, Africa, Asia and the Pacific. The WHO launched a major eradication effort in the 1950s based on mass screening and treatment with injectable penicillin. The campaign examined some 300 million individuals of whom 50 million were treated. Although yaws was not eradicated, by the end of the major campaign in 1964, the burden of yaws had been significantly reduced to ~2.5 million cases.³

Following this initial success of the WHO campaign, yaws dropped down the public health agenda internationally and domestically in many countries. In the 1970s and 1980s, there was a resurgence of cases in some countries in West and Central Africa.^{11,12} This led to a renewal of control efforts, which again reduced the burden of the disease but did not eradicate it.

Over the past 20 years, there has been a further resurgence of yaws in previously endemic countries, and the disease is now thought to be endemic in at least 12 countries in West Africa, Southeast Asia and the Pacific. There are a further 76 countries that previously reported yaws, throughout Africa, the Americas, Asia and the Pacific, for which adequate up-to-date surveillance data are not currently available (Fig. 1).¹⁰ Most yaws cases are concentrated in just three countries: Ghana, Papua New Guinea and the Solomon Islands have each reported >15 000 cases annually within the last 3 years. In another eight countries, transmission occurs in focal communities. Despite being deprioritized in international health fora, both India and Ecuador have reported eliminating yaws in recent years with prolonged campaigns based on case identification,

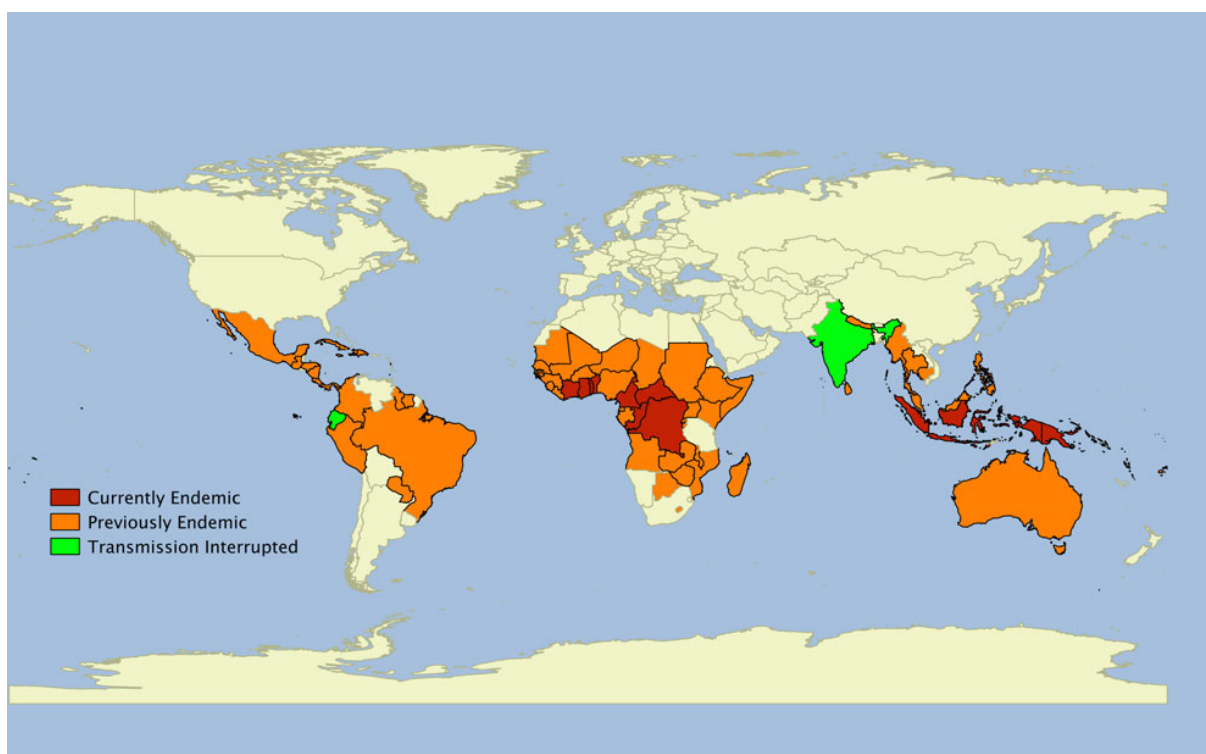


Fig. 1 Worldwide distribution of yaws. Data taken from the World Health Organization. Global Health Observatory Data Repository. <http://apps.who.int/gho/data/node.main.NTDYAWSEND?lang=en> (2 October 2014, date last accessed).

contact tracing and treatment with injectable penicillin,¹³ demonstrating that sustained efforts can be successful.

Bacteriology

Treponema pallidum is a spirochaete that cannot be cultured *in vitro*.⁶ They divide slowly (every 30 h), have a characteristic corkscrew-like motility and can move through gel-like environments such as connective tissue. They are rapidly killed by drying, oxygen exposure or heating, and they cannot survive outside the mammalian host. The four pathogenic treponemes are morphologically and serologically indistinguishable, and share at least 99% DNA sequence homology.¹⁴ Whole-genome sequencing has demonstrated that the genome of *T. p* ssp. *pertenue* differs by only 0.2% from that of *T. p* ssp. *pallidum*,¹⁴ the causative organism of venereal syphilis. The phylogenetic relationship between different subspecies of treponemes is not clear, as very few isolates of the non-venereal subspecies are available.¹⁵

Clinical features

As with other treponemal diseases, the clinical features of yaws may be conveniently divided into primary, secondary and tertiary disease.^{2,6,16} Although this classification is clinically useful, it should be remembered that patients may present with a mixture of clinical signs.

Primary yaws

The initial lesion of primary yaws is a papule appearing at the site of inoculation after ~21 days (range 9–90 days).⁶ This ‘Mother Yaw’ may evolve either into an exudative papilloma, 2–5 cm in size, or degenerate to form a single, crusted, non-tender ulcer (Fig. 2). The lower limbs are the commonest site for primary yaws lesions, but other parts of the body may all be affected. Unlike venereal syphilis, genital lesions are extremely uncommon. In untreated individuals, primary lesions may heal spontaneously over a period of 3–6 months, leaving a pigmented scar.¹⁷ Primary lesions may still be present in patients who present with secondary yaws.

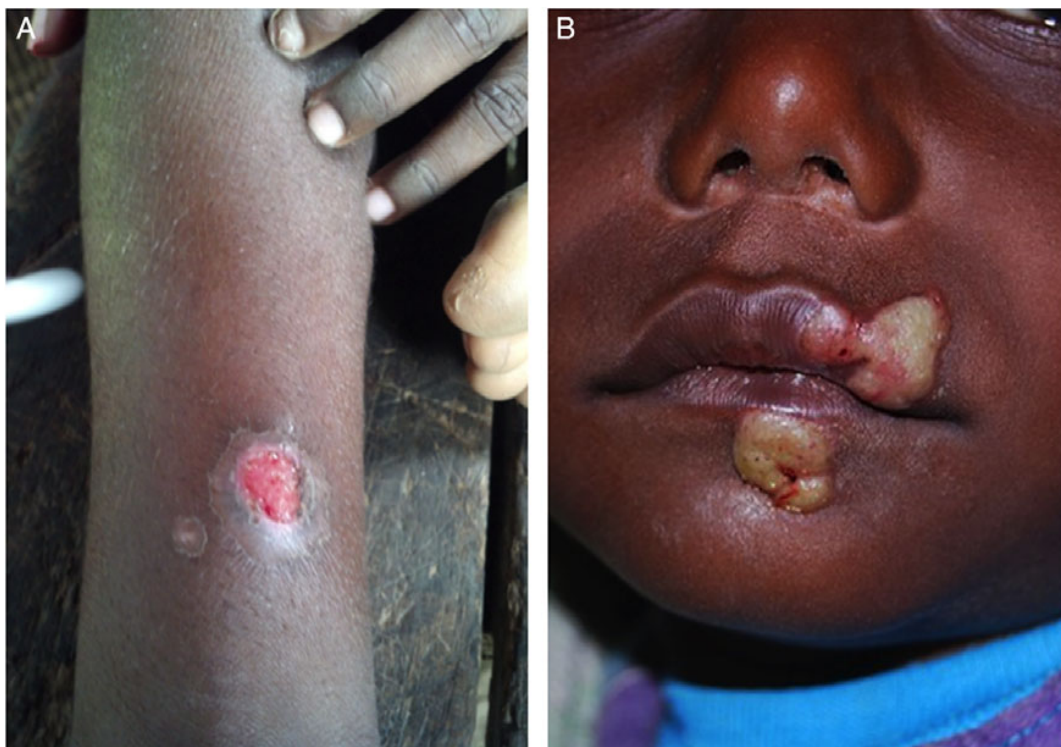


Fig. 2 Lesions of primary yaws. **A**, typical ulcer of primary yaws. **B**, papilloma of primary yaws (Images reproduced with permission of M.M. and O.M.).

Secondary yaws

After a period of 1–2 months (sometimes up to 24 months), haematogenous and lymphatic spread of treponemes may result in progression to secondary yaws, which predominantly affects the skin and bones,^{18,19} often with general malaise and lymphadenopathy.

As with venereal syphilis, a wide range of skin manifestations has been described in secondary yaws (Fig. 3). Patients may develop disseminated papillomatous or ulcerative lesions, scaly macular lesions or hyperkeratotic lesions on the palms and soles.^{6,19} Hyperkeratotic lesions can crack and become secondarily infected, resulting in severe pain and a crab-like gait (crab-yaws).²⁰ Mucous membrane involvement is uncommon in secondary yaws.²¹

Alongside the skin, involvement of the bones is one of the cardinal features of secondary yaws. The most common manifestation is osteoperiostitis, involving the fingers (resulting in dactylitis) or long bones (forearm, fibula and tibia) which results in bony swelling and pain (Fig. 4).¹⁸ In most patients, multiple bones can be affected. In a study from

Papua New Guinea,¹⁹ 75% of children with secondary yaws had joint pain. Following treatment of primary or secondary yaws, skin lesions usually resolve within 2–4 weeks and bone pain may begin to resolve in as little as 48 h.^{1,2}

As in all treponemal infections, untreated patients may develop latent infection, with positive serology but no clinical signs. Latent cases can relapse, usually in the first 5 years (rarely up to 10 years) after infection.⁶ Relapsing lesions tend to occur around the axillae, anus and mouth.

Tertiary yaws

The destructive lesions of tertiary yaws were previously reported to occur in up to 10% of untreated patients but are now rarely seen. As in other stages of the disease, the skin is most commonly affected. Nodular lesions may occur near joints and ulcerate, causing tissue necrosis.⁵ Destructive lesions of the face were one of the most marked manifestations of late-stage yaws. *Gangosa*, a destructive osteitis of the palate and nasopharynx, results in mutilating facial



Fig. 3 Skin lesions of secondary yaws. **A**, Crusted maculopapular lesion of secondary yaws. **B**, Multiple ulcers of secondary yaws (Images reproduced with permission of M.M. and O.M.).



Fig. 4 Bony lesions of secondary yaws. Dactylitis due to secondary yaws (Images reproduced with permission of O.M.).

ulceration. *Goundou*, which was rarely reported even when yaws was hyperendemic, is characterized by exostoses of the maxillary bones.²

Unlike syphilis, yaws is not thought to cause cardiovascular or neurological disease.² Post-mortem studies in a yaws-endemic community in Ghana found that aortitis, histologically similar to that found in tertiary syphilis, was the most common

cardiovascular abnormality, but definitive evidence that this was due to yaws is lacking.

Attenuated disease

The manifestations of yaws appear to be less florid than previously described.²² In particular, tertiary manifestations are now rarely seen. There is no agreed definition of attenuated yaws, nor a clear explanation for why the clinical features of the disease may have changed, although improvements in living standards, use of treponemocidal antibiotics for other infections and mutations in *T. p* ssp. *pertenue* have all been proposed.²²

Diagnosis

Treponema pallidum is not viable *ex vivo*, which has limited the value of direct diagnostic methods. While dark field microscopy allows direct visualization of spirochaetes,⁶ the skills and equipment required are not available in most locations even in relatively high-income settings. Instead diagnosis has rested on

combinations of serological assays and, more recently, nucleic acid amplification tests (NAATs).

As with venereal syphilis, serology has been the mainstay of laboratory diagnosis.²³ Serological detection of yaws requires detection of two distinct antibodies: one against a treponemal antigen and one against a non-treponemal antigen. *Treponema pallidum* particle agglutination (TPPA) and haemagglutination (TPHA) assays are used to detect *Treponema*-specific antibodies. Once positive, these tests usually remain positive for life. The venereal disease research laboratory (VDRL) and rapid plasma reagin (RPR) tests are non-treponemal tests, which may give rise to biological false positives, but more accurately reflect active disease and can be used as a test of cure, since titres fall following successful treatment.

A major challenge for clinicians and epidemiologists has been that the pathogenic treponemes are serologically indistinguishable. Given the considerable epidemiological and clinical overlap between the syndromes, this continues to represent a barrier to accurate data on the incidence and prevalence of yaws.

While serological assays do not require sophisticated equipment, they do require access to laboratory facilities, which are rarely available to the remote communities where yaws is endemic. Rapid diagnostic tests (RDTs) have proved effective in the diagnosis of syphilis,²⁴ and a number of evaluations of their performance in diagnosing yaws have recently been undertaken. In Papua New Guinea, an RDT (Chembio Diagnostic System, Inc., New York, NY, USA) that detects both treponemal and non-treponemal antibodies was shown to be valuable in providing serological confirmation of clinically suspected cases.²⁵ In the Solomon Islands, the same RDT was shown to be of use for community surveillance and mapping.²⁶ These tests may help to improve reporting practices for yaws worldwide.

Polymerase chain reaction (PCR) tests have been commonly adopted for the diagnosis of venereal syphilis, but distinguishing between subspecies of *T. pallidum* currently relies on combined PCR and sequencing²⁷ and is only available at research laboratories. PCR has also emerged as a tool for diagnosing

other causes of skin lesions in yaws-endemic populations, including *Haemophilus ducreyi*.^{28,29} Wider access to NAAT-based diagnostics, including possible point-of-care tests, will be required as part of the WHO yaws eradication programme.

Diagnostic quandaries

The differential diagnosis of tropical ulcerative lesions is broad³⁰ and varies depending on the stage and type of lesion. As examples, the primary lesions of yaws may be mistaken for cutaneous leishmaniasis, tropical ulcer caused by fusobacteria and *Treponema vincentii*, or pyoderma. Of particular importance is the emergence of *H. ducreyi*, the causative organism of the sexually transmitted disease chancroid, as a common cause of non-genital skin lesions in a number of countries where yaws is endemic. There are now reports of *H. ducreyi* as a cause of non-genital skin lesions from Papua New Guinea, the Solomon Islands, Ghana and Vanuatu.^{28,29} Data from experimental models of chancroid suggest that these lesions should be responsive to azithromycin,³¹ and therefore, that mass distribution of azithromycin for yaws (and possibly trachoma) could also be effective in treating lesions resulting from infection with *H. ducreyi*.

Treatment

Long acting, injectable penicillin has been the mainstay of treatment for yaws for over 50 years⁶ and was the cornerstone of all previous yaws control and eradication programmes. Despite extensive use, *T. pallidum* remains exquisitely sensitive to penicillin, with no evidence that resistance has emerged. There have been rare reports of treatment failure following treatment with penicillin, but the difficulty of distinguishing treatment failure from reinfection makes the significance of these findings uncertain.³² The major drawback of injectable penicillin has been the requirement for trained medical staff to administer treatment, the risk of transmitting blood-borne infections and the possible risk of anaphylaxis. The recommended doses of benzathine penicillin for the treatment of yaws (1.2 MU for adults, 0.6 MU for

children) are lower than those used in venereal syphilis,⁶ and in situations where the diagnosis is unclear, clinicians are advised to treat for syphilis.¹⁶

Before 2012, no other agents had been evaluated against yaws in randomized controlled trials, but there are published observational data suggesting prolonged courses of oral penicillin or tetracyclines could be effective.^{33,34} Erythromycin has also been recommended based on its efficacy in the treatment of venereal syphilis. These treatment options are of less relevance since the emergence of azithromycin as an effective treatment for yaws.

The macrolide antibiotic azithromycin was previously shown to be effective in treating venereal syphilis.³⁵ A landmark paper published in 2012 compared a single oral dose of azithromycin (30 mg/kg) to benzathine penicillin in the treatment of primary and secondary yaws.⁴ Azithromycin was non-inferior to penicillin, with clinical and serological cure in 96% of individuals randomized to treatment with azithromycin.

Azithromycin has a number of advantages as an agent in the treatment of yaws. It is orally administered and has a favourable safety profile. It has been widely and successfully used in mass drug administration programmes for the control and elimination of trachoma.³⁶ One area of concern is the possibility of resistance to azithromycin, which is now widespread in sexually transmitted strains of *T. pallidum*.^{37,38} Monitoring for the development of resistance in *T. p* ssp. *pertenue* will be an extremely important component of the WHO yaws eradication strategy.

Eradication efforts

The emergence of azithromycin as an effective, single-dose oral agent for the treatment of yaws has led to renewed interest in the disease. In 2012, the WHO outlined a new strategy (the Morges strategy) for yaws eradication.³⁹ This strategy is based on community mass treatment with single-dose oral azithromycin, with subsequent clinical case detection to direct further rounds of mass or targeted treatment with azithromycin. The WHO is aiming to eradicate the disease by 2020.³⁹

The previous WHO programmes in the 1950s to 1960s resulted in significant reductions in the worldwide burden of yaws³ but did not successfully eradicate it. It is thought that a failure to adequately identify and treat contacts and latent cases alongside a lack of integration of yaws surveillance in to national health programmes were responsible for this failure.⁴⁰

Both India and Ecuador have reported eliminating yaws since 2000, and their experiences are informative for the current global eradication campaign. Ecuador experienced a large drop in yaws incidence and prevalence following the initial WHO campaigns of the 1950s,¹³ but further control efforts were complicated by the anecdotal nature of case reporting. Ecuador instituted a more sustained surveillance programme in the late 1980s, combining continuous village-level monitoring for skin lesions with formal surveys conducted every 5 years that included clinical and serological screening. Individuals identified as having yaws (active or latent) were treated with injectable benzathine penicillin. In surveys conducted between 1988 and 1993, the prevalence of yaws dropped by over 90%, and in a follow-up survey conducted in 1998, no new cases were been detected.¹³

In India, initial eradication efforts were launched in the 1950s, but the disease rebounded in the 1970 and 1980's. In 1996, the government launched a yaws eradication programme.⁴¹ As in Ecuador, the programme involved a combination of clinical screening and treatment with intermittent serological surveys. A notable aspect was the provision of financial incentives for individuals to report suspected cases to the control programme.⁴¹ As in Ecuador, the programme drove a sustained reduction in yaws incidence from 3571 cases in 1996 to 0 cases in 2004.

Despite optimism surrounding mass distribution of azithromycin, there remain major barriers to a successful eradication programme. Notably, there are limited accurate epidemiological data from many countries where yaws is currently reported. Most national surveillance systems report clinically suspected cases only, without serological or PCR confirmation. Given the wide range of phenotypically

similar skin lesions that may occur, it is likely that these figures are inaccurate. Capacity building to support improved surveillance is a central component of the current eradication campaign. This is compounded by the absence of recent data from many countries where yaws was previously reported to be endemic.¹⁰ Significant investment in mapping and epidemiology will be required to go along with the eradication programme. Given the ambitious timescales involved, innovative approaches combining mapping with other NTD or disease mapping activities should be considered.

The eradication programme also lacks dedicated funding and a drug donation programme. The cost of eradication has been estimated to be in the region of \$112 million dollars, excluding drug costs, with a cost of ~\$21 per DALY saved⁴² that compares favourably to many other public health interventions. As data from a larger number of previously endemic countries become available, it is possible that the total cost of the eradication programme may be revised upwards substantially, although efficiencies of scale may bring the cost per DALY down.

Yaws is known to be endemic in several countries where trachoma is also found, including the Solomon Islands and Vanuatu, and co-ordinated mapping efforts have been undertaken or proposed in some of these countries. As both diseases are controlled by mass distribution of azithromycin, and there is already a dedicated drug donation scheme for trachoma elimination, the possibility of integrating yaws and trachoma control efforts warrants further study. Particular attention will need to be paid to the efficacy of the lower dose of azithromycin (20 mg/kg) used in trachoma control programmes for the treatment of yaws.

Conclusions

Yaws remains a problem for poor, rural communities in many countries and places a significant burden on national health-care systems. As with the other treponemal diseases, it has a multi-stage disease course predominantly involving the skin and bones. Yaws remains sensitive to penicillin, but azithromycin has emerged as an effective treatment

that will be the cornerstone of the WHO-led eradication efforts, providing that it can be delivered to the populations that need it.

The eradication of yaws will require considerable support from partners across the health and development sectors, and a number of challenges need to be overcome for this effort to be successful. Accurate epidemiological data are lacking from both currently and formerly endemic countries, and a significant investment to improve this situation is urgently needed. The development and validation of near-patient and laboratory tests specific for treponemal subspecies are urgently required, in view of the increasing recognition that other bacteria can cause phenotypically indistinguishable skin lesions. Integration of these tools, and monitoring for the emergence of macrolide resistance, will be of critical importance as the programme moves forward. Despite these concerns, significant progress has been made in the last 5 years, raising hopes that yaws eradication may finally become possible.

Authors' contributions

M.M. wrote the first draft of the manuscript and made subsequent revisions. O.M., A.W.S., K.B.A. and D.C.M. reviewed and edited the manuscript. M.M. is the guarantor of the paper. K.B.A. and A.W.S. are staff members of the World Health Organization. The author alone is responsible for the views expressed in this article and they do not necessarily represent the decisions or policies of the World Health Organization.

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Conflict of interest

The authors have no potential conflicts of interest.

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Chapter 2: Overview of PhD objectives and study design

2.1 Project summary

Yaws is a re-emerging endemic treponemal infection[1,2] and is recognized by WHO as a Neglected Tropical Disease (NTD). The Pacific Islands are believed to be a major focus of yaws worldwide, though accurate population-based data on infection prevalence are lacking from most countries[2]. In a randomized controlled trial published in 2012, azithromycin at a dose of 30mg/kg (max 2g) was shown to be non-inferior to the previous standard of care, benzathine-penicillin, with a cure rate of 96% for primary and secondary yaws[3]. Based on this finding, WHO developed a strategy for worldwide yaws eradication by 2020, based predominantly on community mass treatment with azithromycin[4].

Mass treatment with azithromycin is also key to the “SAFE” strategy for trachoma elimination[5]; however the dose used in trachoma mass treatment (20mg/kg – max 1g) is lower than that which was demonstrated to be effective against yaws. Although resistance has not been documented in yaws, azithromycin resistance, mediated by point mutations in 23s rRNA, has emerged in other treponemal infections, notably syphilis[6–8]. Both trachoma and yaws are believed to be endemic in a number of countries, in particular in the Pacific region[9]. In areas where trachoma and yaws are co-endemic, mass treatment of populations as part of trachoma control programmes might aid yaws eradication efforts, but could also have negative consequences if drug resistance were to be encouraged.

The aim of this PhD was to answer several questions about the epidemiology and control of yaws. A central component was a series of cross-sectional surveys conducted in the Solomon Islands, which were used to provide insights in to the epidemiology of yaws and assess the impact of community mass treatment with azithromycin, conducted as part of a trachoma control programme, on yaws. It was intended that these data would provide particular insights into the potential effectiveness of a lower dose of azithromycin for treating yaws.

The samples and data collected during these surveys formed the basis of the other major components of this PhD – a validation of a rapid diagnostic serological test for yaws

and a mathematical modelling study designed to understand the programmatic requirements for yaws eradication.

2.2 Aims and objectives

2.2.1 Objectives

1. Establish accurate data on epidemiology of yaws in Solomon Islands
2. Identify risk factors in pre-MDA setting for both yaws disease and infection
3. Assess the impact of azithromycin MDA on yaws prevalence
4. Identify risk factors for treatment failure following MDA
5. Evaluate diagnostic tests for use in yaws eradication programmes
6. Identify programmatic requirements for yaws eradication programmes

2.2.2 Specific aims

1. To examine a population-based sample of individuals for clinical signs of disease and capture their clinical phenotype accurately
2. To use a population-based survey methodology to establish risk factors for disease at an individual and community level
3. To evaluate the effect of mass drug treatment by mapping the distribution of active disease and infection at follow-up, post mass drug administration
4. To establish the sensitivity and specificity of a rapid diagnostic serological test for yaws in a community surveillance setting
5. To build a mathematical model of yaws transmission dynamics and investigate, using the model, the predicted impact of MDA on transmission

2.3 Hypotheses

1. Household and community level factors will be associated with risk of yaws in a pre-MDA environment
2. Treatment with 20mg/kg of azithromycin will effectively treat yaws resulting in a decreased prevalence of active and latent yaws in the community

3. Failure to participate in MDA will be one of the predominant risk factors for yaws in a post-MDA environment
4. Diagnostic tests developed for syphilis will have adequate test characteristics for use in yaws eradication campaigns
5. Thresholds for interruption of yaws transmission will be definable using mathematical modelling

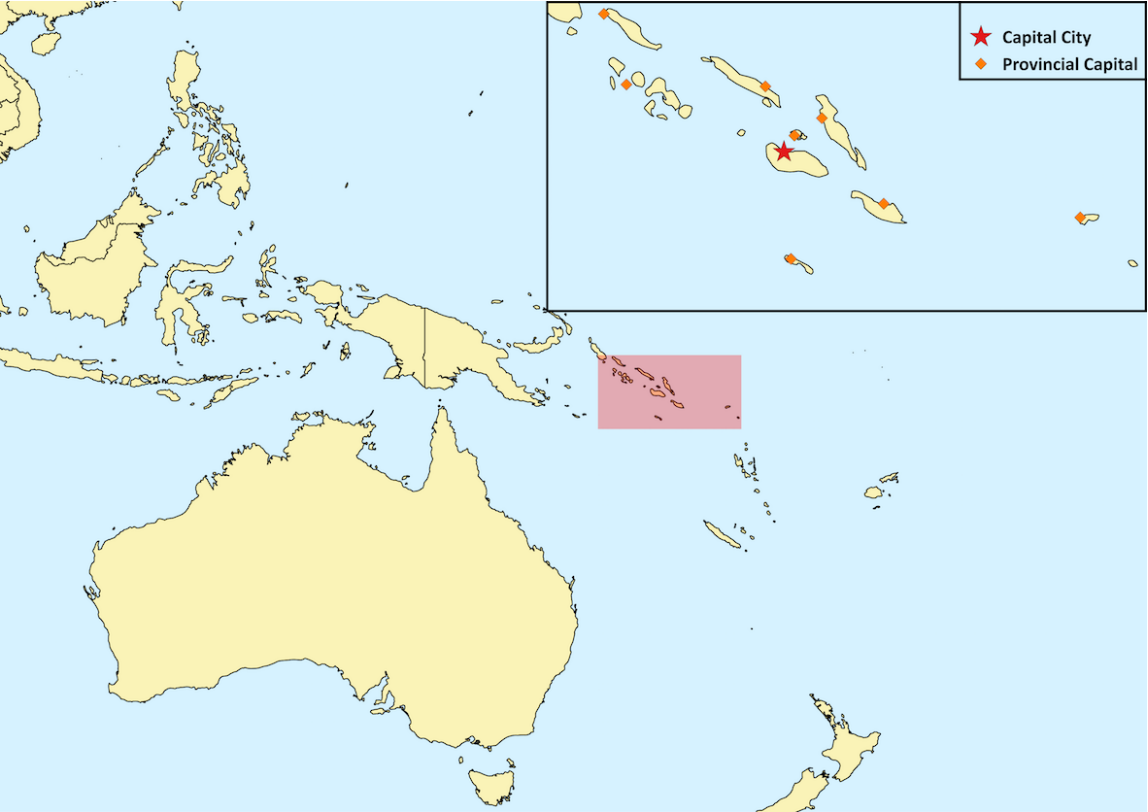
2.4 Study site

2.4.1 The Solomon Islands

The Solomon Islands is an archipelago of approximately 1,000 islands in the Pacific, with a population at the time of the 2009 census of 515,870 [10]. Administratively, the country is divided into ten provinces, including the capital city, Honiara. The majority of the population live in rural communities with only approximately 20% of individuals residing in an urban area. In rural communities most people are subsistence farmers although logging, mining and tourism are sources of income in some settings.

The mean temperature is 27°C with minimal seasonal variation, and the average annual rainfall is between 3,000 and 5,000 mm per year[11]. Tropical cyclones typically occur between November and April. The country is ranked 156th in the world by the Human Development Index [12] with a life expectancy at birth of 67.9 years and a gross national income per capita of \$1,540. Healthcare is provided free at the point of care by the Solomon Islands Ministry of Health and Medical Services (MHMS). Most healthcare is provided by rural health clinics and nurse aid posts staffed by registered nurses and nurse aids. These clinics normally have access to either microscopy or rapid diagnostic tests for malaria, but no other diagnostic facilities. Each province is served by at least one provincial hospital where basic diagnostic tests, including blood tests, are normally available. Referral services are located at the National Referral Hospital in the capital, Honiara.

Figure 1: Map of the Solomon Islands



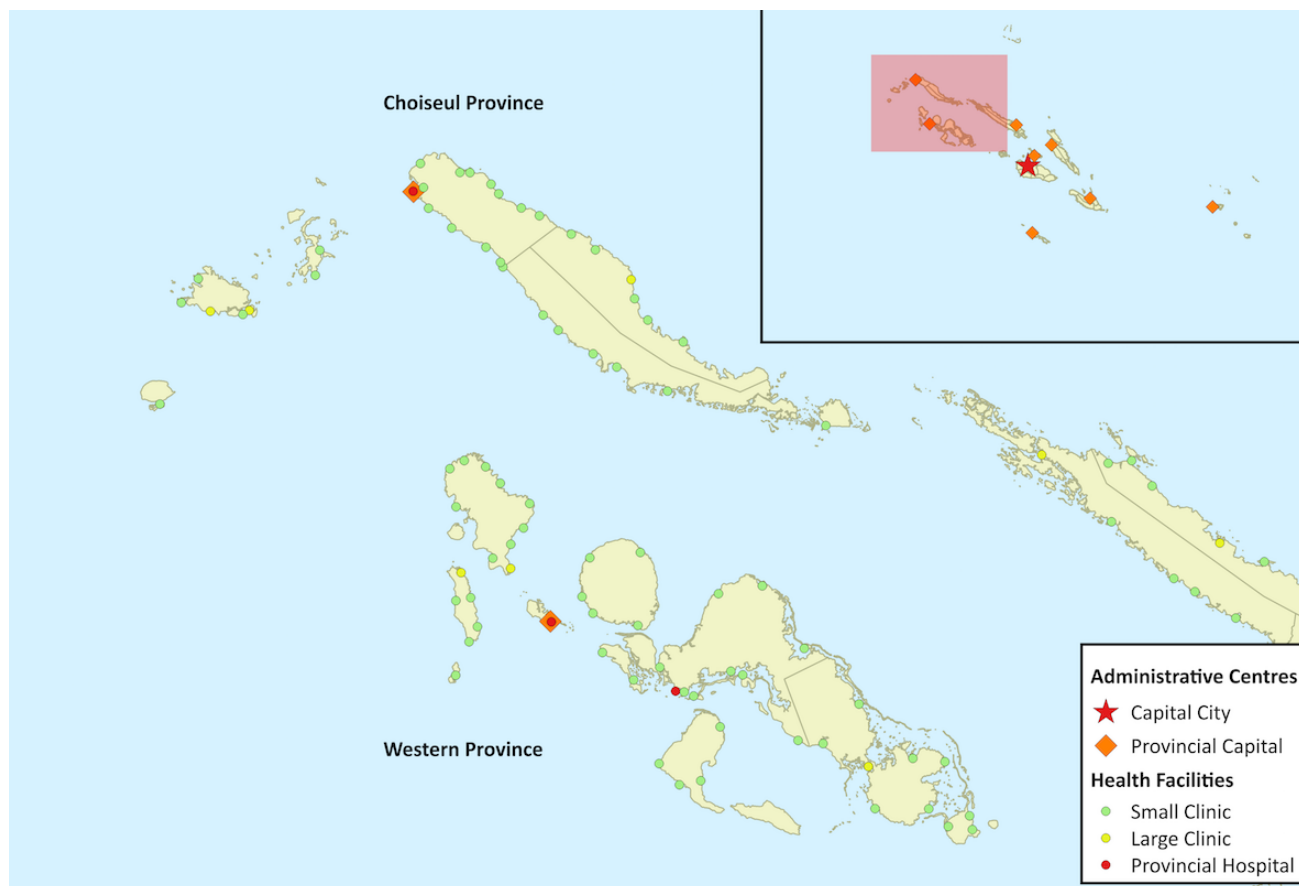
2.4.2 Western and Choiseul province

The fieldwork for this PhD was conducted in Western and Choiseul provinces of the Solomon Islands. These are the northernmost provinces of the country and are close to Bougainville in Papua New Guinea.

Western Province is an archipelago made up of a number of distinct island groups. The administrative capital is Gizo and the province is served by two hospitals and a number of clinics (Figure 3). At the time of the last census, the population of Western Province was 76,649 individuals.

Choiseul Province is comprised of one major island and a small number of surrounding islands. The administrative capital is Taro and the province is served by one hospital and a number of clinics (Figure 3). At the time of the last census the population of Choiseul Province was 26,372 individuals.

Figure 2: Map of Western and Choiseul provinces



2.4.3 Yaws in the Solomon Islands

Yaws is endemic in the Solomon Islands and the country reported 17,043 cases in 2011 (Table 1 and 2). Previous surveys, conducted in the 1980s in the Western Province had reported a prevalence of active primary and secondary yaws of 8.5%[13]. Since that study, no further epidemiological surveys had been conducted in the Solomon Islands. The high number of cases of yaws places a substantial burden on the health system and results in substantial morbidity.

Table 1: Absolute number of reported yaws cases by age group and province, 2011

Province	1-4 yrs	5-14yrs	15-49yrs	50yrs+	Total
Central Islands	71	237	180	60	548
Choiseul	86	475	248	48	857
Guadalcanal	384	1159	768	215	2526
Honiara	180	571	360	59	1170
Isabel	30	177	134	13	354
Makira/Ulawa	201	569	323	78	1171
Malaita	546	1541	1042	282	3411
Renbel	21	144	64	27	256
Temotu	181	544	288	115	1128
Western	1068	3089	1206	259	5622
Solomon Islands	2840	8506	4613	1156	17043

(Data from Solomon Islands MHMS, Health Information System)

Table 2: Reported yaws cases per 1,000 population by age group and province 2011

Province	1-4 yrs	5-14yrs	15-49yrs	50yrs+	Total
Central Islands	19	35	15	21	22
Choiseul	22	67	21	17	33
Guadalcanal	27	48	17	27	27
Honiara	24	44	10	13	19
Isabel	8	27	11	4	14
Makira/Ulawa	30	54	21	12	29
Malaita	26	41	17	19	25
Renbel	55	173	52	48	85
Temotu	62	97	30	39	53
Western	97	158	33	31	74
Solomon Islands	38	64	18	22	33

(Data from Solomon Islands MHMS, Health Information System)

2.4.4 Trachoma in the Solomon Islands

Trachoma, caused by ocular infection with *Chlamydia trachomatis*, is also endemic in the country. The WHO SAFE strategy [5] for the elimination of blinding trachoma combines surgery for trichomatous trichiasis (S), azithromycin mass drug administration (MDA) (A), facial cleanliness (F) and environmental improvement (E).

Current WHO guidelines for trachoma recommend that a minimum of three annual rounds of azithromycin MDA, at a dose of 20mg/kg, should be conducted in districts in which the prevalence of trichomatous inflammation—follicular (TF) is greater than 10% in the 1-9 year old population.

Between 2011 and 2013, the Solomon Islands MHMS completed baseline prevalence surveys for trachoma in all ten provinces of the country. These surveys demonstrated that the prevalence of TF was above the threshold for undertaking MDA in nine of ten provinces. These provinces were therefore due to undertake three annual rounds of azithromycin MDA between 2014 to 2016. The prevalence of TF in Choiseul province was found to be below the threshold for conducting azithromycin MDA, according to international guidelines then in use, and therefore only the other elements of the SAFE strategy were recommended for implementation in this province.

Table 3: Prevalence of trachoma by province in the Solomon Islands

Province	Year of Prevalence Survey	TF Prevalence (%)*	TT Prevalence (%)^	Recommendations for SAFE Intervention
Central Islands	2011	19.2	1.5	Full SAFE intervention
Choiseul	2013	7.0	0.0	- Implement S, F & E. - Sub-district survey is needed
Guadalcanal	2012	15.3	0.0	Full SAFE Intervention
Honiara	2012	12.0	0.0	Full SAFE Intervention
Isabel	2012	24.3	1.2	Full SAFE Intervention
Makira/Ulawa	2012	22.3	0.0	Full SAFE Intervention
Malaita	2012	19.2	0.0	Full SAFE intervention
Western	2013	23.0	0.3	Full SAFE Intervention
Temotu	2013	25.0	0.0	Full SAFE Intervention
Renbel	2013	25.0	0.0	Full SAFE Intervention

(Data from Solomon Islands MHMS)

*In children aged 1-9 years

^ In individuals aged > 14 years

2.5 Study components:

The project consisted of four main components, which aimed to address the six objectives of the PhD.

1. Epidemiology of yaws pre-MDA (*Objectives 1 & 2*)
2. Evaluation of impact of azithromycin MDA on yaws (*Objectives 3 & 4*)
3. Evaluation of point-of-care diagnostic tests in yaws (*Objective 5*)
4. Modelling of yaws transmission and interventions (*Objective 6*)

An overview of each component of the PhD is given below. Detailed introductions and methodologies for each component are reported in the respective Research Papers addressing each objective.

2.5.1 Epidemiology of yaws

(Detailed methodology and results reported in Research Paper – Chapter 3)

There are limited data on the risk factors for yaws infection and disease at a community and individual level. The most recent survey data from the Solomon Islands are from the 1980s [13] but national reporting data and recent studies from Papua New Guinea and Vanuatu suggest that the prevalence of yaws remains high in the Pacific [14,15]. Whilst rainfall and temperature have been reported to be important factors in the distribution of yaws worldwide [16,17] there are extremely limited data on risk factors for infection and disease within endemic populations.

This element of the PhD aimed to broaden our understanding of the pre-MDA epidemiology of yaws in the Solomon Islands and to identify risk factors for infection and disease.

The fieldwork for this component of the study was a cross-sectional survey conducted in Western and Choiseul provinces of the Solomon Islands in 2013. An outline of the fieldwork methodology for the study is given below (*Section 2.6 – Fieldwork Protocol*). Logistic

regression was used to assess for associations at both the individual and household level. At the individual level, age, gender, clinical phenotype (including presence of skin and bone lesions), and reported treatment history were considered. At the household level, household size, WASH variables, and presence of contacts with infection or disease were considered. For both infection and active disease, unadjusted and adjusted odds ratios, controlling for potential confounders, are presented. Clustering was adjusted for at the level of the study cluster using robust standard errors.

2.5.2: Evaluation of azithromycin MDA on yaws

(Detailed methodology and results reported in Research Paper – Chapter 4)

The WHO yaws eradication strategy is based on community mass treatment with azithromycin[4]. This element of the PhD aimed to assess whether MDA, using lower dose azithromycin, conducted for trachoma control, would be effective in reducing the prevalence of yaws and whether it might be sufficient to interrupt transmission. The fieldwork for this component of the study was two cross-sectional surveys conducted in Western provinces of the Solomon Islands in 2014 and 2015 following azithromycin MDA, conducted by the Solomon Islands MHMS.

This component of the PhD used two approaches to measure the impact of azithromycin MDA on prevalence and transmission of yaws at the community level. Firstly a nested case-control design was used, in the initial post-MDA cross-sectional survey, by comparing the seroprevalence of yaws between individuals receiving MDA and individuals not receiving MDA. This methodology has been used before to measure the impact of public health interventions, such as bed net distribution, at the individual level[18]. Secondly, children aged <5 years were included in the second post-MDA surveys. If transmission had been interrupted by MDA then individuals would remain seronegative for infection. Testing of this age group is specifically recommended by WHO to assess for interruption of transmission[4].

Logistic regression was used to calculate unadjusted and adjusted odds ratios for risk factors for both infection and disease. At the individual level, demographic data, as well as household (contact) and village (contacts) level data, alongside MDA compliance data (individual, household and village coverage) were used to assess risk-factors for treatment failure / disease post-MDA. Clustering was adjusted for at the level of the study cluster, using robust standard errors.

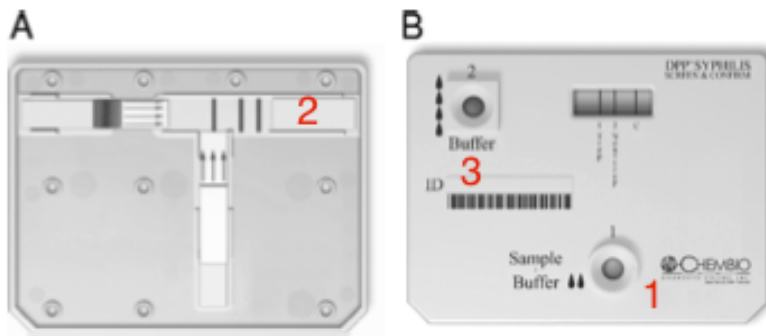
2.5.3 Diagnostics: Validation of Chembio-DPP for use in yaws

(Detailed methodology and results reported in Research Paper – Chapter 5)

Serology has remained the mainstay of laboratory testing for yaws [19]. As with syphilis, laboratory diagnosis of yaws is based on detection of two distinct sets of antibodies: one against a treponemal antigen and one against a non-treponemal antigen. The *Treponema pallidum* particle agglutination (TPPA) and haem-agglutination (TPHA) assays detect *Treponema*-specific antibodies. These tests are more specific than the non-treponemal tests but once positive, they usually remain positive for life. Commonly used non-treponemal tests include the venereal disease research laboratory (VDRL) and rapid plasma reagin (RPR) tests. These are less specific than the treponemal antibody tests but more accurately reflect active disease, with titres rising in active infection and falling following successful treatment.

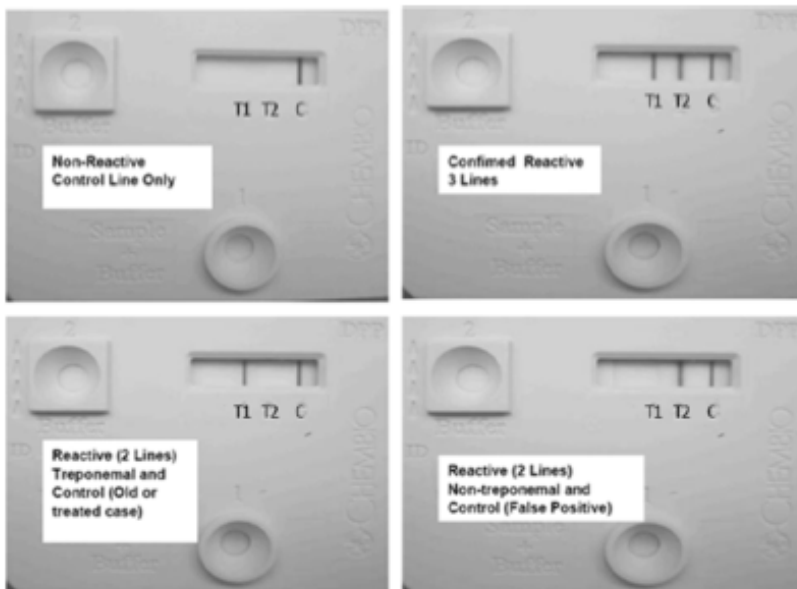
Access to these tests remains limited in many of the remote, rural communities where yaws is found, suggesting that a point of care test may be of value. Syphilis and yaws are indistinguishable using standard serological approaches [20] and therefore a rapid diagnostic test developed for syphilis could potentially provide a valuable point-of-care diagnostic tool in countries where yaws is endemic. The majority of rapid diagnostic tests for syphilis detect only treponemal antibodies and cannot distinguish between current and past infection. Such tests are therefore not suitable for use during a yaws eradication campaign. A novel point of care test platform (the Dual Path Platform, DPP) was developed by the CDC [21] for the detection of both treponemal and non-treponemal antibodies in patients being tested for syphilis (Figures 4 and 5). The platform has been shown to be both highly sensitive (92-95%) and specific (95-99%) for the diagnosis of syphilis [22,23], as compared to traditional serology.

Figure 3: Internal structure of the Dual Path Platform-Syphilis test kit



Sample and running buffer are added at point 1 and migrate to the detection strip 2. If treponemal or non-treponemal antibodies are present they bind a recombinant *T.pallidum* antigen and a non-treponemal antigen respectively. Additional running buffer is added at point 3. This causes colloidal gold-particles conjugated to protein-A and anti-IgM antibodies to migrate into the detection strip. If antibodies to treponemal and non-treponemal antigens are present, visible lines appear within 15 minutes. Typical results of the RDT are shown in Figure 5.

Figure 4: Typical results from the Dual Path Platform-Syphilis test kit



Samples collected as part of the pre-MDA survey were utilized for the DPP-RDT validation

study component of this PhD. TPPA and quantitative RPR were considered the 'gold standard' diagnostic test. The sensitivity and specificity for the DPP-syphilis kit was calculated for the presence of treponemal antibodies and non-treponemal antibodies. For the purposes of this study, simple random sampling was used to select samples to undergo parallel testing with both the DPP kit and traditional serology.

2.5.4: Programmatic requirements for yaws eradication

(Detailed methodology and results reported in Research Paper – Chapter 6)

The current WHO yaws eradication strategy is based on an initial round of total community treatment (TCT) followed by subsequent regular active case finding surveys and treatment of any cases identified and their contacts (Total Targeted Treatment, TTT) [4]. Once zero incident cases are detected, annual surveys are proposed for a period of three years, including serological surveillance of children aged under 5 years. If no incident cases are detected during this period then yaws elimination will be declared. There are limited empirical data to inform the optimum number of rounds and coverage of mass treatment and case finding that will be required to interrupt transmission of yaws and make eradication a possibility.

A cost-effectiveness analysis conducted by WHO suggested that implementation of the yaws eradication strategy was likely to be cost-effective with a cost per DALY averted of \$324 [24]. This analysis assumed that initial treatment coverage would be close to 100% and that transmission would be interrupted by a limited number of rounds of high coverage treatment. As noted, there are limited empirical data to support these assumptions. Firstly, data from Papua New Guinea indicates that even within a research setting, an initial round of mass treatment followed by active case finding is not sufficient to completely interrupt transmission[25]. Secondly, data from a wide variety of other NTD programmes show that high MDA coverage (>90%) is rarely achieved at the programmatic level [26]. Mathematical models have proven effective in providing guidance on the likely programmatic requirements for other NTD programmes and could provide similarly useful insights into the scale up of yaws eradication efforts.

For this component of the PhD, a stochastic Markov model of community-level transmission of yaws was developed. Community mass treatment interventions were modelled in line with the WHO eradication strategy, varying the coverage and number of rounds of community mass treatment. Each combination of disease and intervention parameters was simulated 1,000 times and the eradication probability was defined as the percentage of runs within each combination of model characteristics where interruption of transmission was achieved. Sensitivity analysis was used to explore how the elimination probability was affected by variation in both treatment and disease parameters.

2.6 Fieldwork protocol

The data for the first two components of this PhD were derived from community level surveys in the Solomon Islands, both preceding and following azithromycin MDA. The MDA itself was conducted by the Solomon Islands MHMS as part of the national trachoma control programme.

An overview of the fieldwork methodology used in these surveys is provided below. Detailed methodologies of the fieldwork are provided in the Research Papers (*Chapters 3 and 4*), which report on the findings of these studies.

The pre-MDA surveys were conducted in both Western province and Choiseul province alongside national trachoma prevalence surveys. Independent of the PhD, the pre-MDA prevalence of Trachomatous Inflammation – Follicular (TF) in 1-9 year old children was found to be greater than 10% in Western province and below 10% in Choiseul province. In line with then-current WHO recommendations, azithromycin MDA was therefore only indicated in Western Province and the post-MDA follow-up surveys were conducted there.

2.6.1 Community sensitization

Prior to commencing the surveys conducted during this study, the Solomon Islands MHMS organized a radio broadcast announcing the survey and outlining which regions were involved. In each community, discussions were held with village leaders and community members. Knowledge and opinions on both yaws and, at the baseline survey trachoma, were discussed, and the aims of the study and subsequent MDA programme outlined. The study was also explained to household heads, participating individuals and their parents/guardians. Opportunities for questions to be answered were given at every stage of the process. The sensitization process was conducted by local health staff in appropriate local languages for each community.

2.6.2 Participant inclusion and exclusion criteria

Inclusion Criteria

Baseline survey

Children aged 5-14 years in the target population in whom informed consent was obtained from a guardian and assent was obtained from the child.

6 Months follow-up survey

Children aged 5-14 years in the target population in whom informed consent was obtained from a guardian and assent was obtained from the child.

18 Months follow-up survey

Children aged 1-14 years in the target population in whom informed consent was obtained from a guardian and assent was obtained from the child.

Exclusion Criteria: All surveys

Any participant who declined to participate or was unable to consent or participate in sample collection due to

- Illness
- Incapacity
- Inability to communicate

Exclusion from the study did not affect an individual's participation in the azithromycin MDA programme being undertaken as part of the MHMS trachoma elimination programme.

2.6.3 Informed consent

For all fieldwork, initial consent for the community to participate in the study was obtained from the village elders. For each participating household, the household head provided verbal consent for collection of household level data. Where the household head was absent

at the time of the study visit, the most senior person identified by household members was asked to provide consent. For skin examination and sample collection, the household head or senior adult was asked to provide written consent on behalf of children in the household. Children were also asked to assent to participation in the study. The study aims and protocols were explained to participants by nursing staff fluent in the local dialect. All consent forms were witnessed by a member of the study team. Where consent was not obtained, households or individuals were excluded from the study.

2.6.4 Data and sample collection

Surveys were undertaken between October 2013 and November 2015. Participant level data were collected on demographics (age and gender) and clinical features of yaws. For each included individual, the field team completed a standardized history and examination, collecting information on bone and joint symptoms, yaws treatment history and the number, location, and duration of skin lesions. Information about skin lesions was captured in a standardized format. All information was recorded directly into a purpose-built, Android smartphone app, to ensure completeness and accuracy of data recorded.

The survey team collected either a serum sample (children aged 5-14 years) or a dried blood spot (children aged 1-4 years) from all individuals, regardless of clinical features, for diagnostic testing. In individuals with skin lesions, the team collected lesion swabs using a standardized protocol developed by collaborators at the CDC [27]. Clinical samples were transferred to the National Referral Hospital in Honiara where they were frozen to -20°C. Samples were subsequently transferred on dry ice for diagnostic testing at LSHTM.

2.6.5 Laboratory testing:

In children aged 5-14 years, serum was tested using both TPPA (Mast Diagnostics) and Quantitative RPR (Deben Diagnostics) to establish evidence of both previous (TPPA) and current infection (RPR titre >1/4). In children aged 1-4 years, dried blood spot samples were tested using TPPA alone.

A CDC-developed four-plex real-time PCR was used to establish the presence or absence of treponemal DNA in lesion swabs and to allow differentiation of *T. p. ssp pertenuae* from other pathogenic treponemes [27]. Where treponemal DNA was present, an additional triplex PCR was used to test for the presence or absence of the A2058G and A2059G 23s rRNA gene point mutations that are associated with azithromycin resistance [7,8].

2.7 Data analysis tools

Statistical analyses for the epidemiology studies were conducted using STATA 13.1 software (Stata Corporation, College Station, Texas USA) and the R statistical software package 3.0.2 (The R Foundation for Statistical Computing, <https://www.r-project.org>). Mathematical modelling was performed in R using the adaptiveTau package [28]. Details of specific analysis plans for each component of the project are provided in the individual data chapters below.

2.8 Ethical considerations

All elements of the study were conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the Solomon Islands MHMS Research Ethics Committee (HRC 13/10) and the LSHTM Ethics Committee (6358). Written (thumbprint or signature) informed consent was obtained from the parents or guardians of all children participating in the study.

Clinical examination and sample collection were undertaken by experienced, trained medical and nursing staff under supervision of the candidate. Venepuncture and collection of lesion swabs are routinely performed procedures, which cause mild discomfort.

No active intervention was undertaken as part of this study. Azithromycin MDA for trachoma was carried out by the Ministry of Health in line with guidance from WHO. Survey participants with non-yaws skin pathologies were referred to local health services as appropriate. These data have been used to inform the yaws eradication strategies in the Solomon Islands and in other endemic countries.

2.9 Data management

For each survey, each participant was given a unique identifier. These numbers were used to link clinical, demographic, geographic, and laboratory data. All personal identifiers were removed from the dataset before analysis. All fieldwork data were collected directly into the Opendatakit software data collection system [29], which automatically links all non-laboratory data to the individuals' unique identifier. This system performs real-time data validity checks, ensures all data are automatically entered into the database in a predetermined coded format and minimizes missing data. Data from this system are uploaded directly to a secure password-protected, cloud-based server. All data were verified and inconsistencies resolved prior to analysis. Data will be kept for a minimum of seven years after publication (or ten years after specimen collection, if no publication results from the work).

2.9.1 Quality assurance

Quality control measures have been addressed in the relevant sections of the manuscript. These include:

1. Use of ODK Data Entry Forms on Android Smartphones
 - a. Avoids missing data
 - b. Validation checks on data at the time of entry
 - c. Ensures data coded appropriately
2. Standardization of recording of skin lesions
3. Labelling of all samples with a unique identification number to link samples to individuals, photographs and consent forms
4. Laboratory work conducted by operators blinded to clinical data using standardized SOPS

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Chapter 3: Epidemiology of yaws in the Solomon Islands



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Student	MICHAEL MARKS
Principal Supervisor	DAVID MABEY
Thesis Title	ETHIOBIOLOGY OF ZAVS IN THE SOLMONI ISLANDS AND THE IMPACT OF A TRACTION CONTROL PROGRAMME

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?	AMERICAN JOURNAL OF TROPICAL MEDICINE & HYGIENE		
When was the work published?	2015		
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Student Signature: 

Date: 18/04/16

Supervisor Signature: 

Date: 18/4/16

Mapping the Epidemiology of Yaws in the Solomon Islands: A Cluster Randomized Survey

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Abstract. Yaws, a non-venereal treponemal disease, is targeted for eradication by 2020 but accurate epidemiological data to guide control programs remain sparse. The Solomon Islands reports the second highest number of cases of yaws worldwide. We conducted a cluster randomized survey of yaws in two provinces of the Solomon Islands. One thousand four hundred and ninety-seven (1,497) children 5–14 years of age were examined. Clinical signs of active yaws were found in 79 children (5.5%), whereas 140 children (9.4%) had evidence of healed yaws lesions. Four hundred and seventy (470) (31.4%) children had a positive *Treponema pallidum* particle agglutination assay (TPPA). Two hundred and eighty-five (285) children (19%) had a positive TPPA and rapid plasma regain assay. Risk of yaws increased with age and was more common in males. The prevalence of yaws at village level was the major risk factor for infection. Our findings suggest the village, not the household, should be the unit of treatment in the World Health Organization (WHO) yaws eradication strategy.

INTRODUCTION

Yaws is a non-venereal, endemic treponemal infection caused by *Treponema pallidum pertenuis*¹ and is closely related to *T. p. pallidum*, the causative agent of venereal syphilis.² Unlike syphilis, yaws is generally a disease of children³ living in poor, rural settings and is found mostly in warm and humid environments.⁴ Infection predominantly results in lesions of the skin and bone.⁵ Tertiary yaws causes disfiguring lesions of the face but neurological and cardiovascular manifestations (which can appear in advanced syphilis) are not thought to occur. A major campaign in the 1950s was responsible for a significant reduction in the prevalence of disease worldwide, but following the dismantling of vertical control programs, the number of cases subsequently rebounded, and yaws now represents a significant public health problem in Africa, South-East Asia, and the Pacific.^{6–10}

In a single-center study in Papua New Guinea, azithromycin was shown to be equivalent to benzathine-penicillin in the treatment of both primary and secondary yaws.¹¹ The discovery of an orally effective agent prompted the World Health Organization (WHO) to plan a renewed effort to eliminate yaws using community mass treatment.¹²

Despite optimism, clinical and epidemiological data on yaws remain sparse. Many countries report only clinical cases without laboratory confirmation and there are inadequate data to guide control programs in many countries. Previous studies have shown that the majority of clinical cases occur in children < 15 years of age, and have also suggested a preponderance of cases in males.^{3,8,13} It is also recognized that for every clinical case there are 5–6 individuals with serological evidence of infection but no clinical manifestations.^{8,13}

The Solomon Islands, a Pacific archipelago of ~100 inhabited islands, is endemic for yaws, and in 2010 the country reported 20,635 cases of yaws, the second highest number of cases in the

world after Papua New Guinea, which lies immediately to the north.⁵ We conducted a cluster randomized community survey in two provinces of the Solomon Islands to establish the prevalence of yaws infection and disease, and to explore risk factors associated with infection and disease at community level.

MATERIALS AND METHODS

Participant recruitment. The study was undertaken in Western and Choiseul Provinces of the Solomon Islands in September and October 2013, alongside programmatic trachoma mapping activities.¹⁴ The two provinces were considered as two separate evaluation units. We defined clusters either as villages or as groups of villages to obtain 50 clusters of approximately equal size. Twenty-five clusters were selected at random in each province. In each cluster, a complete census of all households was obtained and 30 households were selected using simple random sampling. In selected households children 5–14 years of age were invited to participate. Where selected households were unoccupied, an alternative household was randomly selected. Written informed consent was obtained from the head of each household by staff fluent in the local dialect. Assent was obtained from all children. We calculated that a sample size of 1,022 would allow us to detect a prevalence of infection of 10% with absolute precision of 3% assuming a design effect of 2.5.

Data collection. Household-level data were collected on location (recorded using the smartphone global positioning system) and Water, Sanitation and Hygiene (WASH) variables, including source of water and the presence or absence of hand washing facilities and latrines.

Participant-level data were collected on demographics and clinical features of yaws. The survey team completed a standardized history and examination, collecting information on bone and joint symptoms, yaws treatment history, and the number, location, and duration of skin lesions. Skin lesions were classified using the WHO pictorial yaws classification scheme. All data were collected using the LINKS software package on Android smartphones.¹⁵

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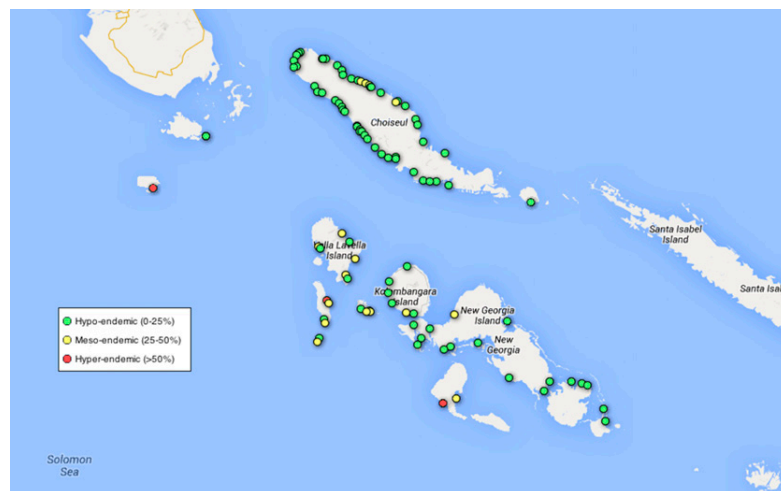


FIGURE 1. Map showing locations of surveyed villages. Villages where the seroprevalence of yaws was 0–25% (hypo-endemic), 25–50% (meso-endemic), and > 50% (hyper-endemic) are shaded green, yellow, and red, respectively. The predominance of coastal villages reflects the distribution of communities within the Solomon Islands.

The survey team performed venipuncture on all individuals, regardless of clinical features, and obtained a serum sample for diagnostic testing. Samples were transferred to the National Referral Hospital in Honiara where they were frozen. Samples were transferred on dry ice for diagnostic testing at the London School of Hygiene & Tropical Medicine (LSHTM).

Laboratory studies. Sera were tested using both the *Treponema pallidum* particle agglutination assay (TPPA, Mast Diagnostics, Merseyside, UK) and a quantitative rapid plasma regain test (RPR, Deben Diagnostics, Ipswich, UK), at LSHTM, by an operator masked to clinical findings.

Ethics. Ethical approval for the study was granted by the ethics committees of the Ministry of Health and Medical Services in the Solomon Islands (HRC 13/10) and LSHTM (6358) in the UK.

Diagnosis. A positive TPPA was taken as evidence of previous or current infection. A positive TPPA combined with a quantitative RPR titer of $\geq 1/4$ was taken to represent dual-positive serology, suggestive of active infection. Individuals with a single ulcerative or papillomatous lesion and dual-positive serology were considered to have primary yaws. Individuals with skin or bony lesions consistent with secondary yaws and dual-positive serology were considered to have secondary yaws. Individuals with late manifestations (gondou, gangosa, or gummatous lesions) would have been considered to have tertiary yaws. Individuals with dual-positive serology but no current clinical signs were considered to have latent yaws.

Analyses. Logistic regression was used to estimate unadjusted and adjusted odds ratios (ORs) for factors associated with both TPPA positivity or dual seropositivity. We classified household size as less than or greater than the national average (5 residents) according to the 2009 census. There are no established criteria for classifying yaws endemicity based on serological findings. We adjusted previous treatment criteria, based on the number of clinical cases,³ by a factor of 5 to accommodate individuals with latent infection. For the purpose of analysis villages were classified as hypo-endemic (0–25% of surveyed individuals dual seropositive), meso-endemic (26–50% of individuals dual seropositive), or hyper-endemic (> 50% of individuals dual seropositive). Robust

standard errors were used to calculate all confidence intervals (CIs) and *P* values to account for village-level clustering. All analyses were performed using Stata 13.1 (StataCorp, College Station, TX).

RESULTS

Clinical findings. One thousand four hundred and ninety-four (1,494) households in 981 villages were visited; there was no-one home, or no residents 5–14 years of age, in 656 households. One thousand four hundred and ninety-seven (1,497) children were enrolled from 98 villages and 838 households (Figure 1). Demographic data on these individuals are shown in Table 1. More children were enrolled in Western province ($N = 889$) than Choiseul province ($N = 608$). Skin lesions, of any type, were found in 290 children (19.4%, 95% CI 14.3–25.6%).

In total, clinical signs suggestive of active yaws were found in 79 (5.5%, 95% CI 4.0–7.5%) children. Of these, 50 children (3.3%, 95% CI 2.4–4.6%) had skin lesions consistent with primary yaws, whereas 29 (2.0%, 95% CI 1.1–3.7%) had signs consistent with secondary yaws. Skin lesions consistent with secondary yaws were found in 19 (1.3%, 0.6–2.9%), bone lesions consistent with secondary yaws were found in

TABLE 1
Demographics

	Western Province	Choiseul Province
Number of children	889 (59.4%)	608 (40.6)
Number of households	494	344
Number of villages	45	53
Number clusters	25	25
Age (median, IQR)	9 (7–12)	9 (7–11)
Male	438 (49.3%)	323 (53.1%)
Household size (median, IQR)	5 (4–6)	5 (4–6)
Number positive TPPA individuals	334 (37.6%)	132 (21.9%)
Number dual seropositive individuals	186 (20.9%)	63 (10.4%)
Village classified as endemic	12 (26.7%)	3 (5.7%)
Village classified as highly endemic	4 (8.9%)	2 (3.8%)
Access to hand washing facilities	137 (15.4%)	52 (8.6%)

IQR = interquartile range; TPPA = *Treponema pallidum* particle agglutination.

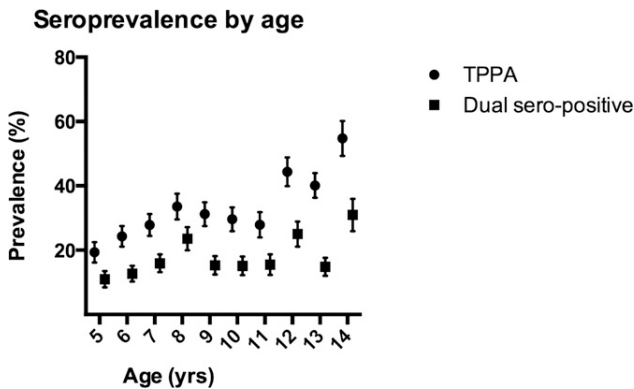


FIGURE 2. Seroprevalence by age prevalence (and 95% confidence interval [CI]) of *Treponema pallidum* particle agglutination (TPPA) assay and dual seropositivity (TPPA and rapid plasma regain [RPR] titer $\geq 1/4$) by age group.

10 (0.7%, 95% CI 0.3–1.5%) children. One hundred and forty (140) children (9.4%, 95% CI 6.1–14.1%) had evidence of healed yaws lesions, of whom 7 also had evidence of active yaws. Two hundred and eighteen (218) children (15.2%, 95% CI 11.0–20.5%) reported treatment of yaws in the last 12 months.

Serological findings. Four hundred and seventy children (470) had a positive TPPA, giving a seroprevalence of previous or current treponemal infection of 31.4% (95% CI 23.6–41.5%). As expected, the prevalence of TPPA positivity increased with age (Figure 2). In Western province, seroprevalence was 37.6% (95% CI 28.1–51.1%), as compared with 21.7% (95% CI 10.4–40.2%) in Choiseul province; after adjustment for clustering this difference was not statistically significant ($P < 0.09$).

Of individuals with a positive TPPA, 285 (60.6%) had an RPR $\geq 1/4$, suggesting active treponemal infection. Dual seropositivity was therefore 19.0% overall, but varied significantly between Western Province ($N = 186$, 21.7%, 95% CI 14.6–30.9%) and Choiseul Province ($N = 63$, 10.4%, 95% CI 4.1–24.2%) ($P = 0.11$).

Twenty-four (30.4%) of 79 children with signs of active yaws and 40 (28.6%) of 140 children with signs of healed yaws

were dually seropositive, compared with 14.8% of children without signs of active or healed yaws ($P < 0.01$ for both comparisons) (Table 2).

Based on serology data we classified 77 (78.6%) villages as non-endemic, 15 (15.3%) villages as endemic, and 6 (6.1%) villages as highly endemic. Eighty percent of dual-serologically positive individuals lived in a meso-endemic or hyper-endemic village.

Risk factors. In both unadjusted and adjusted analyses, age, sex, number of dual seropositive household contacts, degree of village endemicity, and access to hand washing facilities were all associated with both TPPA positivity and dual seropositivity, and active disease. Household size was not a risk factor for either infection or active disease (Tables 3 and 4).

DISCUSSION

This study confirms that yaws is endemic in the north-west of the Solomon Islands, with one-third of children in this area showing evidence of infection by TPPA. In keeping with national case reporting, we observed a difference in the prevalence of clinical signs of yaws and of both TPPA positivity and dual seropositivity between Western and Choiseul provinces, although this result was not statistically significant after accounting for clustering. As anticipated, there was significant clustering of both infection and disease. The degree of endemicity at village level was the strongest risk factor for both TPPA positivity and dual seropositivity, a finding that suggests communities, not households, should be the target of contact tracing and treatment as part of the WHO yaws eradication campaign in this population. Treatment failure with penicillin has previously been reported to be associated with the degree of endemicity at village level.¹⁶ Our data support the theory that reinfection, rather than true treatment failure, may partially explain this finding.

Seropositivity was strongly associated with the number of seropositive household contacts. It is possible that this association is a consequence of transmission to household contacts from an actively infected individual rather than a risk factor for initial infection per se. Longitudinal data would be needed to better explore this association.

TABLE 2
Serology results

		Overall	Western Province	Choiseul Province
Clinical evidence of primary yaws	n	50	38	12
	TPPA positive/RPR negative	1 (2%)	1 (2.6%)	0 (0%)
	TPPA positive/RPR $< 1/4$	3 (6%)	2 (5.3%)	1 (8.3%)
Clinical evidence of secondary yaws	TPPA positive/RPR $\geq 1/4$	17 (34%)	11 (29.0%)	6 (50%)
	N	29	28	1
	TPPA positive/RPR negative	2 (6.9%)	2 (7.1%)	0 (0%)
Clinical evidence of previous yaws	TPPA positive/RPR $< 1/4$	3 (10.3%)	3 (10.7%)	1 (0%)
	TPPA positive/RPR $\geq 1/4$	7 (24%)	6 (21.4%)	1 (100%)
	N	140	135	5
No clinical evidence of active yaws	TPPA positive/RPR negative	21 (15%)	21 (15.6%)	0 (0%)
	TPPA positive/RPR $< 1/4$	25 (17.9%)	24 (17.8%)	1 (20%)
	TPPA positive/RPR $\geq 1/4$	40 (28.6%)	40 (29.6%)	0 (0%)
No clinical evidence of active yaws	N	1278	688	590
	TPPA positive/RPR negative	59 (4.6%)	28 (4.1%)	31 (5.3%)
	TPPA positive/RPR $< 1/4$	122 (9.5%)	75 (10.8%)	47 (8.0%)
	TPPA positive/RPR $\geq 1/4$	188 (14.8%)	132 (19.2%)	56 (9.5%)

TPPA = *Treponema pallidum* particle agglutination; RPR = rapid plasma regain.

TABLE 3
Risk factors for TPPA positivity

		Illustrative prevalence data	Unadjusted OR	Adjusted OR
Age (years)*		5–9: 27.0% 10–14: 37.9%	1.14 (1.08–1.20) $P < 0.001$	
Sex		Male: 35.1% Female: 28.6%	1.35 (1.11–1.64) $P = 0.002$	
Household size		≤ 5: 29.8% > 5: 35.6%	1.30 (0.90–1.88) $P < 0.160$	
Number of household dual seropositive contacts†	0 1 > 1	22.9% 69.1% 86.1%	7.52 (4.72–12.0) $P < 0.001$ 20.80 (9.25–46.76) $P < 0.001$	3.92 (2.16–7.12) $P < 0.001$ 10.74 (4.38–26.37) $P < 0.001$
Household dual seropositive contacts with skin ulcers‡		Absent: 30.7% Present: 82.4%	10.54 (3.43–32.33) $P < 0.001$	7.52 (4.72–12.0) $P < 0.001$
Village§	Hypo-endemic Meso-endemic Hyper-endemic	14.5% 67.0% 74.2%	12.02 (6.99–20.68) $P < 0.001$ 17.01 (9.50–30.46) $P < 0.001$	10.22 (6.35–16.42) $P < 0.001$ 20.51 (12.11–34.75) $P < 0.001$
Hand washing facilities¶		Absent: 33.2% Present: 23.4%	0.62 (0.35–1.09) $P = 0.095$	0.62 (0.35–1.09) $P = 0.095$

*Relative increase in odds per year increase in age.

†TPPA positive with a reactive RPR titer $\geq 1/4$. Adjusted for age, gender, household size, and village endemicity.

‡TPPA positive with a reactive RPR titer $\geq 1/4$ and an ulcerative skin lesion. adjusted for age, gender, household size, and village endemicity.

§Adjusted for age, gender, household size, and household contacts.

¶Hand washing facilities reported by family within 15 m of toilet/latrine. Adjusted for age, gender, and household size.

OR = odds ratio; TPPA = *Treponema pallidum* particle agglutination; RPR = rapid plasma regain.

As in previous studies, there were several individuals with serological evidence of infection for every individual with clinically apparent disease,^{8,13} and a large number of individuals with typical skin lesions who were not dually seropositive.¹⁷ Lesions not associated with positive serology may reflect early yaws before conversion, recent treatment of yaws with declining RPR titers, misclassification of the lesion by survey personnel, or alternative aetiologies of ulcerative skin lesions. *Haemophilus ducreyi* has recently been identified as a possible causative agent of yaws-like lesions in the Pacific region.^{18–20}

As expected, the prevalence of TPPA positivity increased with age within the age group examined, and the study also confirmed previous reports^{8,13} that males are at slightly higher risk than females of both TPPA positivity and dual seropositivity. The reasons for this association are unclear. It is possible that the association with gender reflects an earlier onset of sexual intercourse in boys than girls, with misclassification of

cases of syphilis as yaws in endemic communities. In our study, the association with gender remained when analyses were restricted to children < 10 years of age, making this kind of misclassification less likely.

An unexpected finding was the protective association between access to hand washing facilities and the risk of TPPA positivity and dual seropositivity. It is thought that yaws is spread by skin-to-skin contact when bacteria from a lesion enter by a breach in the skin.^{3,21} It is plausible that access to hand washing facilities promotes better overall skin health, reducing the number of small abrasions and lesions, which might serve as portals of entry for *T. pallidum* ssp. *pertenue*. It is also possible that this association is explained by residual confounding, from better general living conditions or access to health care facilities.

Although we did not examine adults during this survey, we did not see any cases of tertiary yaws in the communities we visited, a finding in keeping with a previous yaws survey in the

TABLE 4
Risk factors for dual seropositivity

			Unadjusted OR	Adjusted OR
Age*		5–9: 15.4% 10–14: 19.1%	1.08 (1.02–1.14) $P = 0.006$	1.08 (1.03–1.14) $P = 0.003$
Sex		Male: 19.5% Female: 14.4%	1.44 (1.38–1.83) $P = 0.002$	1.48 (1.17–1.88) $P = 0.001$
Household size		≤ 5: 16.0% > 5: 18.9%	1.22 (0.79–1.90) $P = 0.362$	
Number of household dual seropositive contacts†	0 1 > 1	9.9% 46.4% 59.3%	7.85 (4.12–14.97) $P < 0.001$ 13.20 (6.40–27.21) $P < 0.001$	4.18 (2.12–8.25), $P < 0.001$ 6.74 (2.72–16.70), $P < 0.001$
Household dual seropositive contacts with skin ulcers‡		Absent: 16.2% Present: 52.9%	5.82 (2.57–13.21) $P < 0.001$	7.52 (4.72–12.0) $P < 0.001$
Village§	Non-endemic Endemic Highly endemic	4.95% 40.11% 51.6%	12.85 (7.17–23.02) $P < 0.0001$ 20.46 (10.79–38.82) $P < 0.0001$	8.30 (5.36–12.86) $P < 0.001$ 20.33 (11.88–34.80) $P < 0.001$
Hand washing facilities¶		Absent: 18.4% Present: 8.0%	0.39 (0.16–0.90) $P = 0.029$	0.39 (0.16–0.91) $P = 0.029$

*Increase in odds per year increase in age.

†TPPA positive with a reactive RPR titer $\geq 1/4$. Adjusted for age, gender, household size, and village endemicity.

‡Adjusted for age, gender, household size, and village endemicity.

§TPPA positive with a reactive RPR titer $\geq 1/4$ and an ulcerative skin lesion. adjusted for age, gender, household size, and village endemicity.

¶Hand washing facilities reported by family within 15 m of toilet/latrine. Adjusted for age, gender, and household size.

TPPA = *Treponema pallidum* particle agglutination; RPR = rapid plasma regain.

Solomon Islands.¹⁷ The reasons why late stage manifestations are less common than previously reported are unclear, but improved access to health care and use of antibiotics with treponemocidal activity for other infections may partially account for this finding.

In summary, our data confirm that yaws is highly endemic in the Solomon Islands. Our study confirms previously suggested associations between gender and infection while also highlighting the need for further studies to examine the association with access to hand washing facilities. Prevalence of disease at a village level was shown to be the major risk factor for disease at an individual level, a finding that suggests the community should be the unit of treatment in response to the identification of cases of yaws, in the WHO yaws eradication strategy.¹² Further studies are needed to investigate whether the associations shown in this study remain after community mass treatment with azithromycin.

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Principal Supervisor	DAVID MAREY
Thesis Title	EPIDEMIOLOGY OF YERSINIA IN THE SOLOMON ISLANDS AND THE IMPACT OF A TRAINING INTERVENTION PROGRAMME

If the Research Paper has previously been published please complete Section B, if not please move to Section C

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***Haemophilus ducreyi* Associated with Skin Ulcers among Children, Solomon Islands**

Michael Marks, Kai-Hua Chi, Ventis Vahi, Allan Pillay, Oliver Sokana, Alex Pavluck, David C. Mabey, Cheng Y. Chen, and Anthony W. Solomon

During a survey of yaws prevalence in the Solomon Islands, we collected samples from skin ulcers of 41 children. Using PCR, we identified *Haemophilus ducreyi* infection in 13 (32%) children. PCR-positive and PCR-negative ulcers were phenotypically indistinguishable. Emergence of *H. ducreyi* as a cause of nongenital ulcers may affect the World Health Organization's yaws eradication program.

Bacterial ulcerative skin diseases are a common cause of illness in the developing world (1). Some of these diseases, including Buruli ulcer, caused by *Mycobacterium ulcerans*, and yaws, caused by *Treponema pallidum* subspecies *pertenue*, occur only in tropical and subtropical climates. Yaws is endemic in the Solomon Islands, where ≈15,000 cases per year are reported (2). In 2012, the World Health Organization (WHO) launched a worldwide yaws eradication program based on treatment by mass distribution of azithromycin and monitoring for skin ulcers (3).

Reports suggest that *Haemophilus ducreyi*, the causative organism of chancroid, a sexually transmitted infection, may be associated with nonsexual transmission of nongenital ulcers of the skin in persons from the Pacific region (4,5). If this organism is a common cause of skin ulcers in the region, this factor has crucial implications for the yaws eradication strategy. PCR has been shown to be highly sensitive and specific for diagnosing chancroid (6). We used real-time PCR to detect *H. ducreyi* in skin ulcer samples collected during a survey for yaws in the Solomon Islands.

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The Study

We conducted a cross-sectional survey for yaws in the Western Province and Choiseul Province of the Solomon Islands in 2013. In each province, we chose 25 clusters using a probability-proportionate-to-size method. In each cluster, we selected 30 houses by random sampling; children 5 to 14 years of age living in those houses were invited to participate. Informed written consent was obtained from the children's parents.

Children underwent standardized examination. We recorded location, classification, and duration of skin lesions and yaws treatment history using the LINKS system (7, <http://www.linkssystem.org/>). Lesions were classified by using the WHO pictorial grading scheme for yaws (8). Tenderness was classified based on reports by children. Blood samples were collected from all children. For children with exudative skin lesions, a sample for PCR was collected by rolling a sterile cotton-tipped swab across the lesion and placing it in a cryotube pre-filled with 1.2 mL of AssayAssure solution (Thermo Fisher Scientific, Waltham, MA, USA). Samples were transferred to Honiara National Referral Hospital within 5 days and stored at -20°C . Serum samples were placed on dry ice and shipped to the London School of Hygiene & Tropical Medicine and lesion samples to the US Centers for Disease Control and Prevention.

Serum samples were tested by using *T. pallidum* particle agglutination (Mast Diagnostics, Merseyside, UK) at the London School of Hygiene & Tropical Medicine. For samples with a positive *T. pallidum* particle agglutination, a rapid plasma regain test was performed (Deben Diagnostics, Ipswich, UK). DNA was extracted from lesion samples in a CDC laboratory by using iPrep PureLink gDNA blood kits and the iPrep purification instrument (Life Technologies, Grand Island, NY, USA). A real-time duplex PCR targeting the DNA polymerase I gene (*polA*, *tp0105*) of pathogenic treponemes (which detects all 3 *T. pallidum* subspecies) and the human *RNase P* gene (to monitor for PCR inhibition) was performed by using a Rotor-Gene-Q real-time PCR instrument (QIAGEN Inc., Valencia, CA, USA) (9). Negative (no-template) control and positive controls for *T. pallidum* DNA were included in each PCR run. Considering reports of *H. ducreyi* and the occurrence of *M. ulcerans* in Papua New Guinea, immediately north of the Solomon Islands, we performed a second duplex real-time PCR for *M. ulcerans* and *H. ducreyi* on all samples by using previously validated targets (10,11).

For the purpose of analysis, lesions were classified as acute (<4 weeks) or chronic (>4 weeks). A rapid plasma regain titer $\geq 1/4$ was considered positive. Fisher exact test was used to compare characteristics of patients whose lesions contained *H. ducreyi* with patients whose lesions did not contain *H. ducreyi*. Analyses were performed by using STATA 13.1 (<http://www.stata.com/>).

During the survey, 1,497 children were examined. Samples for PCR were collected from 41 children who had exudative lesions (19 male, median age 8 years). Twenty-two children had ulcerative lesions from which a sample could not be collected. Twelve (29.3%) children had positive results for yaws from serologic testing, but no DNA evidence of *T. pallidum* subsp. *pertenue* or *M. ulcerans*, causative organisms of yaws and Buruli ulcer, respectively, was detected in any sample. *H. ducreyi* DNA was amplified from 13 (32%) samples (Figure). PCR inhibitors were not found in any samples. Clinical data were incomplete for 2 participants. There were no notable differences in the recorded characteristics of skin lesions or in the serologic status of patients in whose ulcers *H. ducreyi* DNA was found compared with those in which *H. ducreyi* was not found (Table).

Conclusions

H. ducreyi is frequently present in skin ulcers of children in the Solomon Islands, and lesions containing *H. ducreyi* DNA were similar in location, duration, and tenderness to lesions in which *H. ducreyi* was not found. Papua New Guinea reported a similar finding (12). Experimental models of chancroid have demonstrated that injection of *H. ducreyi* into the epidermis and dermis causes nongenital skin disease, suggesting that *H. ducreyi* may be the cause of some ulcers in our survey (13). Similar to results for experimental models, *H. ducreyi* DNA was found more frequently in samples collected from boys (8/13; $p = 0.179$), although this difference was not statistically significant. It is possible that the difficulty of collecting samples for molecular testing, the lack of facilities to enable collection of samples for culture in affected areas, and the precise culture requirements of *H. ducreyi* have notably delayed recognition of this association (14).

Lesions associated with *H. ducreyi* were found in patients with positive and negative serologic test results for *T. pallidum* subsp. *pertenue*. It is likely that patients with positive serologic test results represent latent yaws with an alternative etiologic agent causing the current lesion. The possibility that there are alternative causes of childhood skin ulcers in the Pacific region could have implications for WHO's yaws eradication strategy, which is based on detection of suspected clinical cases. Although azithromycin is effective in treating genital strains of *H. ducreyi* and experimental



Figure: Example of lesion from which sample was obtained and *Haemophilus ducreyi* DNA was amplified, Solomon Islands, 2013. Photograph ©2014 Michael Marks.

nongenital lesions (15), further studies are needed to confirm efficacy in nongenital lesions in a clinical setting. The emerging data suggest that surveillance strategies should routinely require molecular diagnostics.

A causative agent was not identified in a large proportion of lesion samples. A variety of possible reasons exist for this, including the fact that some lesions were noninfectious, such as insect bites, some numbers of organisms were below current limits of detection of real-time-PCR, or that other organisms, such as staphylococci, for which PCR was not performed, caused these lesions. The sample collection/transport media and PCR assays we used varied from those used by Mitjå et al (12), but it is unclear to what extent this effected our results. A single

Table. Comparison of skin ulcer samples from 41 patients tested for *Haemophilus ducreyi*, Solomon Islands, 2013*

Characteristic	No. (%) samples tested for <i>H. ducreyi</i> by real-time-PCR, 95% CI		p value
	Positive, n = 13	Negative, n = 28	
Male sex	8 (62), 32%–86%	10 (36), 19%–56%	0.179
Location of lesion on leg	12 (92), 64%–99%	21 (96), 80%–99%	0.561
Duration <4 weeks	7 (54), 25%–81%	14 (54), 33%–73%	0.632
Painful lesion	8 (62), 32%–86%	17 (65), 44%–83%	0.542
Sample TPPA-positive	6 (46), 19%–75%	17 (61), 41%–78%	0.503
Sample TPPA- and RPR-positive	5 (38), 14%–68%	7 (25), 11%–45%	0.469

*TPPA, *Treponema pallidum* particle agglutination; RPR, rapid plasma regain test.

sample was collected per patient, but several patients (n = 8, 19.5%) had >1 skin lesion. Swabbing every lesion may have increased the diagnostic yield for *H. ducreyi* and/or *T. pallidum* subsp. *pertenue*. Further studies to explore causes of skin ulcers in this community are needed to better inform disease control efforts.

Because it was not anticipated that *H. ducreyi* DNA would be found in nongenital skin lesions, we did not prospectively collect data on regional lymphadenopathy; however, we did not notice marked lymphadenopathy or bubo formation. Collection of samples for culture and sequencing of the *H. ducreyi* genome are needed to inform our understanding of relatedness of these strains to genital strains.

This study has 2 main limitations. First, the number of samples tested was small. Second, lesion samples were tested for only 3 organisms, raising the possibility that other organisms caused a large proportion of skin ulcers. Despite these limitations, this study clearly demonstrates that *H. ducreyi* is frequently present in childhood skin ulcers in this yaws-endemic community. Further studies of the epidemiology, microbiology, and response to treatment for this newly described pathogen–disease association are required.

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Ethical approval of this study was granted by the Solomon Islands Ministry of Health and Medical Services, the London School of Hygiene & Tropical Medicine in the United Kingdom, and the Centers for Disease Control and Prevention (CDC) in the United States.

Dr Marks is a Wellcome Trust Clinical Research Fellow at the London School of Hygiene and Tropical Medicine. His research focuses on the impact of mass administration of azithromycin on treponemal infections as part of a strategy to eliminate yaws in the Solomon Islands.

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Chapter 4: Impact of azithromycin mass drug administration on yaws



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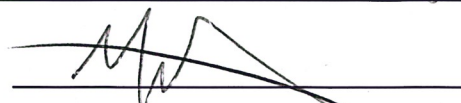
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RESEARCH ARTICLE

Impact of Community Mass Treatment with Azithromycin for Trachoma Elimination on the Prevalence of Yaws

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Abstract

Background

Community mass treatment with 30mg/kg azithromycin is central to the new WHO strategy for eradicating yaws. Both yaws and trachoma—which is earmarked for elimination by 2020 using a strategy that includes mass treatment with 20mg/kg azithromycin—are endemic in the Pacific, raising the possibility of an integrated approach to disease control. Community mass treatment with azithromycin for trachoma elimination was conducted in the Solomon Islands in 2014.

Methods

We conducted a study to assess the impact of mass treatment with 20mg/kg azithromycin on yaws. We examined children aged 5-14 years and took blood and lesion samples for yaws diagnosis.

Results

We recruited 897 children, 6 months after mass treatment. There were no cases of active yaws. Serological evidence of current infection was found in 3.6% (95% CI= 2.5-5.0%). This differed significantly between individuals who had and had not received azithromycin (2.8% vs 6.5%, $p=0.015$); the prevalence of positive serology in 5-14 year-olds had been 21.7% (95% CI=14.6%-30.9%) 6 months prior to mass treatment. Not receiving azithromycin was associated with an odds of 3.9 for infection ($p=0.001$). National figures showed a 57% reduction in reported cases of yaws following mass treatment.

Competing Interests: The authors have declared that no competing interests exist.

Discussion

Following a single round of treatment we did not identify any cases of active yaws in a previously endemic population. We found a significant reduction in latent infection. Our data support expansion of the WHO eradication strategy and suggest an integrated approach to the control of yaws and trachoma in the Pacific may be viable.

Author Summary

Yaws is a bacterial infection closely related to syphilis. The WHO has launched a world-wide campaign to eradicate yaws by 2020. This strategy relies on mass treatment of the whole community with the antibiotic azithromycin. Mass treatment with the same antibiotic is also recommended by WHO to treat the eye condition trachoma but the dose used for this is lower. In this study we assessed the impact of a trachoma control programme in the Solomon Islands on yaws. Following a single round of mass treatment the number of yaws cases fell significantly compared to before treatment. We also saw fewer new cases of yaws being reported to the Ministry of Health. Our findings suggest that mass treatment with the lower dose of azithromycin is also effective as a treatment for yaws. In countries where both yaws and trachoma are found it may be possible to develop an integrated strategy for both conditions.

Introduction

Yaws, caused by *Treponema pallidum* subsp *pertenue*, is a non-venereal infection closely related to syphilis that predominantly affects children living in remote, rural communities in tropical countries[1]. Infection manifests as lesions of the skin, bone and cartilage and, untreated, may progress to destructive tertiary lesions[2]. Yaws was once widespread throughout the tropics. Previous yaws control efforts in the middle of the twentieth century were based on treatment with injectable long-acting penicillin[3], and resulted in significant reductions in the burden of disease worldwide[4]. Despite these initial successes, the disease subsequently rebounded in a number of countries and it is currently thought to be endemic in at least 12 countries across West Africa, South East Asia and the Pacific[1].

In 2012, treatment with azithromycin was shown to be highly effective for yaws[5], and community mass treatment became the foundation of the new WHO Morges yaws eradication strategy[6]. Azithromycin has a number of advantageous characteristics as a mass treatment agent, including oral route of administration, long tissue half-life, and an acceptable side-effect profile.

Community mass treatment with azithromycin is also central to the control of trachoma[7], but the recommended dose used in trachoma control (20mg/kg, max 1g) is lower than that recommended for yaws (30mg/kg, max 2g). The International Task Force for Disease Eradication highlighted the need to investigate the effect of lower dose azithromycin for the treatment of yaws, and the possibility of synergies with trachoma control programmes in countries where the two diseases are co-endemic. In some areas of Ghana in which azithromycin mass drug administration was previously used for trachoma control, yaws is currently undetectable[8], supporting the hypothesis that lower dose azithromycin may be effective.

Unexpectedly, several recent studies have demonstrated that *Haemophilus ducreyi*[9,10] is a common cause of non-genital ulcerative skin lesions in children in yaws endemic communities. This is a finding which can present difficulties for clinical case identification. Community

perceptions of the value of mass treatment campaigns may be affected by the impact of azithromycin on other common skin infections. Genital strains of *H. ducreyi* are responsive to azithromycin[11], so it is possible that mass treatment with azithromycin may have a synergistic benefit on non-yaws ulcerative skin lesions in these communities.

Both yaws and trachoma are endemic in the Solomon Islands[12], which routinely reports the third highest number of cases of yaws among all countries worldwide[13]. In 2014, the Solomon Islands Ministry of Health and Medical Services (MHMS) undertook community mass treatment with azithromycin as part of the SAFE strategy for trachoma elimination. We performed a prospective study in the Western Province of the Solomon Islands to assess the impact of azithromycin used against trachoma on the prevalence of active and latent yaws.

Methods

As previously described [12], in September and October 2013, we conducted a pre-mass treatment survey in Western and Choiseul Provinces of the Solomon Islands, and documented a high prevalence of active and latent yaws. In mid 2014, mass antibiotic treatment was undertaken for trachoma by the Ministry of Health, in Western Province only, Choiseul Province not qualifying for mass treatment on the basis of a lower prevalence of active trachoma. Azithromycin was administered at 20mg/kg (max 1g) with dose determined by body weight, measured using analogue scales. Due to the death of a local religious leader in June 2014, some communities in the province were in mourning and did not receive treatment with azithromycin. For the purposes of this study, we randomly selected a subset of Western Province communities known to have received treatment with azithromycin. At each household, we collected data on number of residents and whether no, some, or all members of the household had received treatment with azithromycin as part of the mass treatment campaign for trachoma. We enrolled children aged 5–14 years for assessment, collecting individual level data on age, gender, the presence or absence of clinical signs and symptoms of yaws, yaws treatment history, and whether the individual reported having received treatment with azithromycin. We categorized skin lesions using the WHO yaws pictorial guide[14]. All data were entered directly into Android smartphones using the ODK software package[15].

Venepuncture was performed and a serum sample collected from each individual. In individuals with ulcerative or papillomatous skin lesions, we also collected a swab sample of lesion exudate. Exudate was transferred to a FTA Elute Micro Card (GE Healthcare, Buckinghamshire, UK) using three firm side-to-side passes of the swab across the card. We placed each card in its own re-sealable plastic packet with a sachet of desiccant. All samples were transferred to the National Referral Hospital, Honiara, where they were frozen at -20°C, and shipped on dry ice to the London School of Hygiene & Tropical Medicine (LSHTM), UK and the Centers for Disease Control and Prevention (CDC), USA for diagnostic testing.

Laboratory testing

Serum samples were tested at LSHTM with the *Treponema pallidum* particle agglutination test (TPPA, Mast Diagnostics, Merseyside UK). On samples that were TPPA-positive, a quantitative plasma reagin test (RPR, Deben Diagnostics, Ipswich, UK) was performed. Lesion swabs were tested at the CDC using a multiplex real-time (RT) PCR for the identification of *Treponema pallidum* sub-species DNA[16]. If the *T pallidum* PCR was positive, we intended to use a second multiplex RT PCR to detect mutations in the 23S rRNA gene associated with azithromycin resistance. Regardless of the result of the *T. pallidum* PCR, we performed an additional duplex RT PCR for the detection of *Haemophilus ducreyi* and *Mycobacterium ulcerans* DNA [9]. All laboratory testing was performed by individuals masked to the clinical findings.

Routine reporting of yaws incidence data

Suspected cases of yaws are reported via the MHMS Health Information System. We extracted data on the number of cases of yaws seen, per month, across all clinics in the Western Province of the Solomon Islands during the period 2011 to 2014 to allow an assessment of the impact of community mass treatment on the incidence of disease presentation.

Statistical analysis

A positive TPPA was taken as evidence of previous or current infection. Individuals with clinical signs of yaws, a positive TPPA and an RPR titre of $\geq 1:4$ (dual-seropositivity) were considered to have active yaws. Individuals without clinical signs of yaws and with a positive TPPA and an RPR titre of $\geq 1:4$ were considered to have latent yaws. An RPR titre of $\geq 1:16$ was considered to be a high-titre positive. We classified household size as ≤ 5 or >5 residents, 5 householders being the national average according to the most recent census[17]. Household treatment with azithromycin was categorized as complete, incomplete (at least 1 individual not treated) or none. The prevalence of active and latent yaws was compared between individuals who had and had not received treatment with azithromycin. Multivariable logistic regression was used to estimate unadjusted and adjusted odds ratios (ORs) for factors associated with both TPPA- and dual-seropositivity. Robust standard errors were used to calculate all confidence intervals (CIs) and P values, to account for village-level clustering[18]. The impact of mass treatment on cases reported to the MHMS was analysed by fitting a linear regression model to the time series on incident yaws cases, controlling for known seasonal variations and trend in yaws incidence. To account for autocorrelation, the error in the model was assumed to follow an autoregressive process, with a lag of one. All analyses were performed using Stata 13.1 (Statacorp, Texas).

Sample size

Our pre-mass drug administration (MDA) survey had shown that the prevalence of dual-seropositivity in these communities was approximately 20%[12]. Assuming that treatment with azithromycin is 90% effective, the prevalence in people who receive treatment would be anticipated to be approximately 2% post treatment. The prevalence of yaws in untreated individuals was also predicted to fall due to reduced community transmission, although there were no data to guide the likely magnitude of this effect. Assuming, conservatively, that prevalence amongst untreated individuals would fall by 25%, 72 individuals receiving azithromycin and 72 individuals who did not receive azithromycin would have 90% power to detect a difference in the prevalence of yaws. Given anticipated community coverage of 90%, a total survey sample of 720 individuals would therefore be required.

Ethical approval

Written informed consent was obtained from each participating child's parent or guardian by a member of staff fluent in the local dialect. Assent was obtained from all children. Ethical approval for the study was granted by the ethics committees of the Solomon Islands MHMS and the LSHTM (6358).

Results

We enrolled 897 children from 441 households in 11 communities. The median age of children was 9 years, and 466 (52%) were male. 717 children (80%) reported having been treated with azithromycin as part of the trachoma control programme (Table 1) (S1 File).

Table 1. Demographics of study subjects.

Number of children		897
Number of households		441
Household size (Median, IQR)		6 (4–7)
Household MDA Coverage	Complete	59%
	Incomplete	28%
	None	13%
Age (Median, IQR)		9 (7–12)
Male		466 (52%)
Received treatment with azithromycin		717 (80%)
Reported treatment for yaws in last year		93 (10%)

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Two hundred and thirty seven children (26%) had a clinically apparent skin lesion. Twenty-eight children (3.1%) had a skin lesion clinically consistent with yaws. Lesions were more common in individuals who had not received MDA, but this difference was not statistically significant. (4.9% vs 2.6%, $p = 0.101$). No individual with a skin lesion consistent with active yaws had dual-positive serology. Bone swelling consistent with secondary yaws was rare, occurring in only 4 subjects (0.5%). Sixty children (6.7%) had skin lesions consistent with healed yaws. Other skin lesions including ringworm and bacterial infections were common (158 children, 17.6%).

Two hundred and twenty eight children (25%, 95% CI 23–28%) had a positive TPPA. The prevalence did not differ significantly between individuals who had and had not received treatment with azithromycin (24% vs 26%, $p = 0.598$). Thirty two children (3.5%, 95% CI 2.5–4.9%) had a positive TPPA and an RPR titre of $\geq 1:4$; the prevalence of this differed significantly between individuals who had and had not received treatment with azithromycin (2.8% vs 6.6%, $p = 0.015$). 11 children (1.2%, 95% CI 0.06–2.2%) had a high titre positive RPR and this also differed significantly between individuals who had and had not received azithromycin (0.8% vs 2.7%, $p = 0.046$) (Fig 1). We collected lesion swabs from twenty individuals. Swabs could not be collected from eight ulcerative lesions as they were dry. No sample tested positive for *T. p* subsp. *pertenue*, but 7 swabs (35%) were positive for *H. ducreyi*.

Given the small number of individuals with dual sero-positivity these subjects were combined into a single group for the purpose of further analysis. People who had not taken azithromycin had higher odds of dual sero-positivity than those who had (OR = 2.49, 95% CI 1.2–5.2, $p = 0.015$), and after adjusting for confounding due to age, gender, and community of residence, the odds ratio was 3.8 (95% CI 1.8–8.5, $p = 0.001$) (Tables 2 and 3). Increasing age was associated with TPPA positivity, but no other variable was associated with dual sero-positivity.

In the pre-mass treatment period ($n = 36$ months) the mean monthly number of cases of yaws reported by clinicians in the Western Province was 184. In the interrupted time series analysis, the number of cases was 183 in the dry season and 158 in the wet season ($p = 0.440$), and mass treatment was followed by a reduction in the mean number of cases reported per month of 101 case (relative reduction 57%, $p = 0.044$) (Fig 2).

Discussion

In this study, a single round of community mass treatment with 20mg/kg azithromycin, given for trachoma elimination, resulted in a significant reduction in the prevalence of both active and latent yaws, from 1.5% and 20.2% pre-treatment [12], to 0.0% and 3.6% post-treatment ($p = 0.002$ and <0.001 , respectively). The prevalence of infection declined both in individuals who had received treatment and in those who had not, suggesting that a single round of

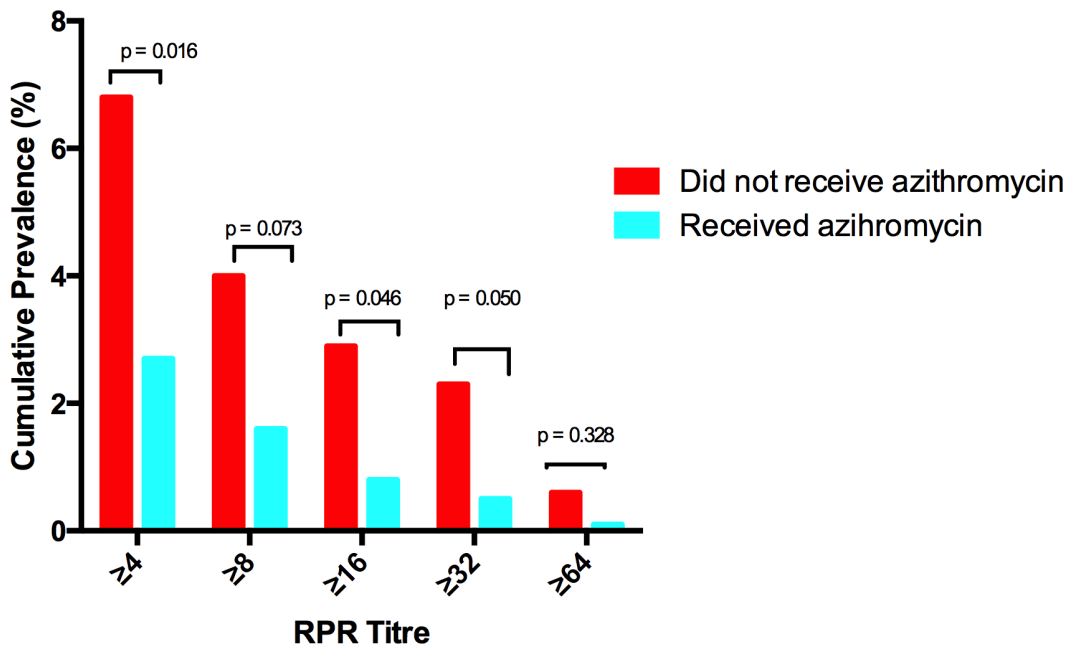


Fig 1. Prevalence of dual sero-positivity in individuals who did and did not receive treatment with azithromycin.

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treatment may have reduced transmission, resulting in a population level benefit that extended to individuals who were not themselves treated. Consistent with this, the impact of azithromycin appeared particularly marked in reducing the prevalence of high-titre positive individuals, who are thought to drive transmission at community level. Our results are mirrored in the routine reporting data for incident yaws cases, which showed a profound drop following mass treatment with azithromycin. There was also a reduction in the prevalence of any ulcerative skin lesion since our previous survey (6.0% vs 3.1%, $p = 0.004$). Taken together, these data suggest that a single round of 20mg/kg azithromycin mass treatment given for trachoma may have interrupted yaws transmission, resulting in a reduction of both prevalent and incident yaws cases, and reducing the prevalence of skin lesions due to other bacteria.

Table 2. Risk factors for TPPA Positivity.

Variable	Indicative prevalence data	Unadjusted Odds Ratio	95% CI	p-value
Age*	5–10: 21% 11–14: 32%	1.1	1.1–1.1	<0.001
Male	Male: 27% Female: 24%	1.2	0.9–1.6	0.247
Household size	≤5: 25% >5: 26%	1.0	0.8–1.4	0.836
Reported taking azithromycin	Yes: 26% No: 24%	0.9	0.6–1.3	0.598
Household MDA	Complete: 25% Incomplete: 25% None: 28%	0.8	0.5–1.4	0.472
		0.9	0.5–1.3	

*Risk associated with a 1 year increase in age

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Table 3. Risk factors for dual sero-positivity.

Variable	Indicative prevalence data	Unadjusted Odds Ratio	95% CI	p-value
Age	5–10: 3%	1.1	0.9–1.2	0.291
	11–14: 4%			
Male	Male: 4%	1.4	0.7–2.8	0.394
	Female: 3%			
Household size	≤5: 4%	0.8	0.4–1.7	0.5980
	>5: 3%			
Reported taking azithromycin	Yes: 2.8%	2.5	1.2–5.2	0.015
	No: 6.7%			
Household MDA	Complete: 3.0%	1.1	0.5–2.6	0.097
	Incomplete: 3.2%			
	None: 6.8%			
		2.4	0.9–5.7	

doi:10.1371/journal.pntd.0003988.t003

The results of this study are concordant with recently published data from Papua New Guinea, which also demonstrated that a single round of azithromycin mass treatment, albeit at a higher dose of 30mg/kg, significantly reduced the prevalence of active and latent yaws [19]. In our study, effectiveness was demonstrated with a lower dose of azithromycin (20mg/kg), evidence that facilitates integration of yaws control into national trachoma elimination plans. The absence of any lesions which were positive by PCR for *T. p* subsp. *pertenue* is consistent with the marked effect seen on serological markers of infection. Our failure to detect treponemal DNA is somewhat reassuring in the context of the theoretical potential for lower dose azithromycin to select for macrolide resistance [20]. Integrated, synergistic control efforts are likely to result in increased efficiencies and decreased costs for programmes and ministries of health, which will be vital in helping countries achieve elimination targets by 2020.

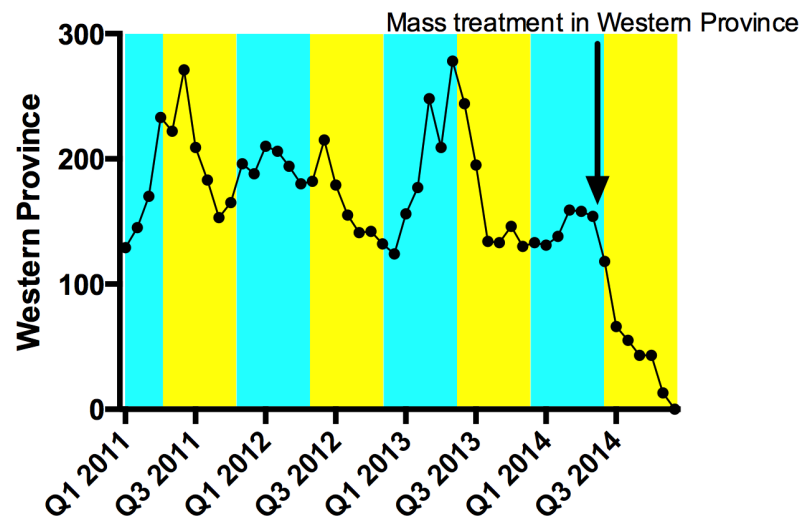


Fig 2. Yaws clinical case reporting in Western Province, 2011–2014. The rainy season is indicated in blue and the dry season in yellow. In the pre-mass treatment period there is evidence of seasonal variation in the incidence of yaws, which is well recognized. Following mass treatment there was a profound drop in the number of cases reported.

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In this population, individual level coverage with azithromycin was about 80%, which is in line with that commonly achieved by trachoma elimination programmes. Our findings suggest that an initial mass treatment round with high coverage can significantly reduce the burden of infection. Whether subsequent treatment would be best delivered through community mass treatment or the detection of cases and contacts remains unclear and should be studied further using both observational and modeling approaches. In view of the extremely low positive predictive value of clinical signs for the diagnosis of yaws seen here, the call for point of care serological tests to be made available within the health care system[21] must be redoubled, in order to strengthen surveillance and guide post-mass treatment case detection and treatment.

In trachoma control programmes in sub-Saharan Africa the use of height-based dosing algorithms commonly results in children receiving doses of azithromycin closer to 30mg/kg than 20mg/kg[22] which might make it difficult to detect meaningful differences in outcomes between the two dosing strategies. As there were limited anthropometric data to guide height-based dosing in the Pacific, weight-based dosing was used in the Solomon Islands, and children therefore received a dose as close as possible to 20mg/kg body weight, to a maximum of 1g. This study therefore provides the first prospective data supporting the effectiveness of lower dose azithromycin against yaws. This information is of particular value for countries where yaws and trachoma are co-endemic and which may therefore benefit from existing trachoma elimination activities.

The most notable limitation of this study is its observational nature. Whilst a randomized design may have been desirable, this would have been unethical, given the need to implement international guidelines for trachoma elimination[23], which mandate treatment of the whole population. A stepped-wedge design could have been considered[24] but may have been unethical, for the same reason. Follow-up in this study was limited to 6 months, and it is possible that longer observation would have revealed a more marked difference between the two groups. We relied on reported receipt of azithromycin, which may have introduced an element of recall bias. However it is likely that this would, in fact, have reduced any difference seen between individuals who did and did not receive azithromycin, and therefore would not affect the overall finding of our study. RPR titres normally fall rapidly in individuals successfully treated for yaws and, in the original randomized control trial of azithromycin conducted in Papua New Guinea[5], combined clinical and serological cure was 96% at 6 months. It seems likely therefore that we have observed the greater part of the effect that might be expected to be derived from azithromycin mass treatment.

Our findings support the roll out of mass treatment with azithromycin as an effective intervention for the simultaneous elimination of trachoma and yaws in co-endemic areas, and provide further observational data to recommend the WHO Morges strategy where yaws alone is endemic. The reduction in the prevalence of latent yaws following community mass treatment is a particularly important result, as a failure to adequately treat these individuals is thought to have contributed to the failure of previous yaws eradication efforts. Community mobilization, ongoing surveillance and lasting political support will be necessary to translate these findings into the ambitious goal of yaws eradication.

Supporting Information

S1 Checklist. Strobe checklist.
(DOCX)

S1 File. Supplementary data file.
(XLS)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: MM VV OS TD CB AWS DCM. Performed the experiments: MM VV OS GK EP KHC AP. Analyzed the data: MM AP CB AWS DCM. Contributed reagents/materials/analysis tools: AP. Wrote the paper: MM VV OS KHC EP GK AP TD CB AWS DCM.

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	1-2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2-3
Objectives	3	State specific objectives, including any pre-specified hypotheses	2-3
Methods			
Study design	4	Present key elements of study design early in the paper	3-4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3-4
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	3-4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	4
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	4
		(b) Describe any methods used to examine subgroups and	N/A

		interactions	
		(c) Explain how missing data were addressed	N/A
		(d) If applicable, describe analytical methods taking account of sampling strategy	4
		(e) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—e.g. numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed	4-5
		(b) Give reasons for non-participation at each stage	4-5
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (e.g. demographic, clinical, social) and information on exposures and potential confounders	4-5 + Table 1
		(b) Indicate number of participants with missing data for each variable of interest	4-5 + Table 1
Outcome data	15*	Report numbers of outcome events or summary measures	5
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included	5
		(b) Report category boundaries when continuous variables were categorized	5 + Table 2 & 3
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—e.g. analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarize key results with reference to study objectives	6-7
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	8
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses,	8

		results from similar studies, and other relevant evidence	
Generalizability	21	Discuss the generalizability (external validity) of the study results	8
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	In submission information

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.



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SECTION A – Student Details

Student	MICHAEL MARICS
Principal Supervisor	DAVID MABBY
Thesis Title	EPIDEMIOLOGY OF ZIKV IN THE SOLMON ISLANDS AND THE IMPACT OF A TROPICAL CURFE PROGRAMME

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?	
When was the work published?	
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	
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Stage of publication	IN PEER REVIEW

SECTION D – Multi-authored work

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Student Signature: 

Date: 19/11/16

Supervisor Signature: _____

Date: _____

Prevalence of active and latent yaws in the Solomon Islands 18 months after azithromycin mass drug administration for trachoma.

Short Title: 18 month follow-up of trachoma MDA on Yaws

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Trachoma

Yaws

Azithromycin

Elimination

Eradication

Abstract:

Introduction:

Both yaws and trachoma are endemic in the Pacific. Mass treatment with azithromycin is the mainstay of the WHO strategy for both the eradication of yaws and the elimination of trachoma as a public health problem, but the dose recommended for trachoma is lower than that for yaws. In countries where both diseases are endemic, there is a potential for synergy between yaws and trachoma control programs, if mass treatment with the lower dose of azithromycin was shown to be effective for the treatment of yaws. In an earlier study we demonstrated a profound reduction in the clinical and serological prevalence of yaws following a single round of mass treatment with azithromycin 20mg/kg undertaken for the purposes of trachoma elimination.

Methods:

This survey was conducted 18 months following a single round of azithromycin mass treatment in the same communities in which we had conducted our previous six month follow-up survey. We examined children aged 1-14 years and took blood and lesion samples for yaws diagnosis using the *Treponema pallidum* particle agglutination assay (TPPA) and the non-treponemal Rapid Plasma Reagin (RPR) test .

Results:

A total of 1,284 children were enrolled in the study. Amongst children aged 5-14 years, 223 had a positive TPPA (27.5%, 95% CI 13.6-47.7%). The TPPA seroprevalence amongst this age group did not differ significantly from either our pre-mass treatment survey or our initial follow-up survey. Thirty-five children had positive TPPA and positive RPR (4.3%, 95% CI 2.1-8.7%), and this did not differ significantly from our initial post-MDA follow-up survey (4.3% vs

3.5%, $p = 0.43$) but remained significantly lower than our initial pre-MDA survey (4.3% vs 21.7%, $p < 0.0001$). Village level MDA coverage was strongly associated with dual-seropositivity ($p = 0.005$). Amongst children aged 1-4 years, 16 had a positive TPPA (3.5%, 95% CI 1.6-7.1%). This did not differ significantly from the seroprevalence in this age group that had been predicted based on our previous surveys (3.5% vs 5%, $p = 0.11$). Fourteen children (1.1%) were considered to have a skin lesion clinically consistent with yaws, but none of these individuals was seropositive for yaws. Of nine cases where a swab could be collected for PCR, all were negative for *Treponema pallidum* subsp. *pertenue* DNA.

Discussion

In this study we have shown that the benefit of a single round of mass treatment with azithromycin 20mg/kg appears to extend to 18 months without any further intervention. The lack of a significant change in seroprevalence from 6 to 18 months after mass treatment might suggest that interventions could be spaced at yearly intervals without a significant loss of impact, and that this might facilitate integration of yaws eradication with other neglected tropical disease (NTD) control programmes. MDA coverage above 90% was associated with significantly better outcomes than coverages lower than this threshold, and strategies to improve coverage at all stages of yaws eradication efforts should be investigated.

Author Summary

Yaws is a neglected tropical disease caused by a bacterium closely related to the agent of syphilis. Mass treatment is recommended by WHO for the control of both yaws and the blinding eye disease trachoma, but the dose used for trachoma is lower (20mg/kg vs 30mg/kg). We have previously shown that a single round of mass treatment with azithromycin for trachoma had a significant impact on the number of cases of yaws in a community, suggesting that the lower dose of azithromycin might be effective for yaws and that trachoma and yaws programmes in the Pacific might be integrated. We repeated our survey 18 months following the initial round of mass treatment to see if the benefit seen at 6 months had persisted. In this study, the number of yaws cases remained significantly lower than before mass treatment even without any additional public health interventions taking place in the 18 months between MDA and this follow-up. This might suggest that annual mass treatment could be used rather than the current recommendation of six-monthly treatment. An annual treatment strategy might facilitate integrating yaws eradication efforts with other NTD control programmes.

Introduction

Yaws is an endemic treponemal disease caused by *Treponema pallidum* subsp *pertenue* [1]. Most cases of yaws are seen in rural communities in tropical countries[2] and are manifest as lesions of the skin, bones and joints. In 2012, single dose azithromycin was shown to be an effective treatment for yaws[3], and mass treatment with azithromycin was subsequently adopted by WHO as the cornerstone of a new yaws eradication campaign [4]. Active yaws is usually seen in children aged 5-14 years, and examination of this age group has been used in many studies to assess the prevalence of active yaws [5–7]. WHO also recommends surveillance by serology of children aged 1-4 years to determine whether transmission has been interrupted [4].

The Pacific is a particular focus for yaws with a large number of cases reported in Papua New Guinea, the Solomon Islands and Vanuatu [2]. Trachoma, caused by ocular infection with *Chlamydia trachomatis*, is also endemic in the Pacific. As community mass treatment with azithromycin is also central to the control of trachoma [8], there is the potential for synergies between trachoma and yaws programmes in the Pacific [9]. A challenge is the difference in the dose of azithromycin recommended for trachoma (20mg/kg, max 1g) compared to yaws (30mg/kg, max 2g). We previously demonstrated that the prevalence of active and latent yaws declined markedly six months after a single round of mass treatment with azithromycin given as part of a trachoma control program in the Solomon Islands [10], providing the first evidence that low dose azithromycin is effective in the treatment of yaws.

The optimal number and timing of rounds of mass treatment needed to interrupt transmission of yaws are unknown [11], although pilot data from other countries suggest

that a single round is inadequate [7]. In 2015 a decision was made not to undertake a second round of azithromycin mass treatment in the Solomon Islands in light of a low reported prevalence of trachomatous trichiasis and a low prevalence of ocular infection with *C. trachomatis* [12]. This provided an opportunity to assess any potential rebound in active and latent yaws in the absence of further control measures. It was hoped that these data might inform expansion of yaws control efforts and, in particular, any potential for synergy between yaws and trachoma control programs in the Pacific.

Methods

This survey was conducted 18 months following a single round of azithromycin mass treatment conducted by the Solomon Islands Ministry of Health and Medical Services. The study was conducted in the same communities in which we had conducted our previous six month follow-up survey [10]. No further rounds of mass treatment (or other specific interventions against yaws) had been conducted in these communities since then.

For each household we collected data on the number of residents. We enrolled children aged 1-14 years for assessment, collecting individual-level data on age, gender, the presence or absence of clinical signs and symptoms of yaws and yaws treatment history. We categorized skin lesions using the WHO yaws pictorial guide [13]. All data were entered directly into Android smartphones using the ODK software package [14].

For children aged 5-14 years, venepuncture was performed and a serum sample collected. In children aged 1-4 years a finger-prick blood sample was collected onto a filter paper. Filter papers were air-dried and stored in sealed bags with desiccant sachets. From individuals with ulcerative or papillomatous skin lesions, we also collected a swab sample of lesion exudate. Exudate was transferred to a FTA Elute Micro Card (GE Healthcare, Buckinghamshire, UK) using three firm side-to-side motions of the swab across the card. Each card was placed in its own re-sealable plastic packet with an individual desiccant sachet. The samples were transferred to the National Referral Hospital in Honiara, where they were frozen at -20°C, and shipped to the London School of Hygiene & Tropical Medicine (LSHTM), UK, and the Centers for Disease Control and Prevention (CDC), USA, on dry-ice for testing.

Laboratory Testing

Blood samples were tested at LSHTM. Filter paper samples were eluted and tested using the *Treponema pallidum* particle agglutination test (TPPA, Mast Diagnostics, Merseyside UK) as previously described [15]. For serum samples a TPPA test was performed initially and for samples that were TPPA-positive, a quantitative rapid plasma reagin test (RPR, Deben Diagnostics, Ipswich, UK) was performed.

Lesion swab samples were tested at the CDC using a number of multiplex real-time (RT) PCR assays. Initially, samples were tested using an assay for the identification of *T. pallidum* subspecies DNA [5]. If the PCR was positive for *T. pallidum* subsp. *pertenue* this was followed by a second multiplex RT PCR to detect mutations in the 23S rRNA gene which are associated with azithromycin resistance. All samples were also tested with a duplex RT PCR for the detection of *Haemophilus ducreyi* and *Mycobacterium ulcerans* [16]. All laboratory testing was performed by individuals masked to the clinical findings.

Statistical Analysis

In children aged 1-4 years, a positive TPPA was taken as evidence of exposure to yaws. For children aged 5-14 years, a positive TPPA was considered as evidence of previous or current yaws infection. We considered individuals with clinical signs of yaws and both a positive TPPA and an RPR titre of $\geq 1:4$ (dual-seropositivity) to have active yaws. We considered individuals who were dual-seropositive but without clinical signs of yaws to have latent yaws. For the purpose of the analysis an RPR titre of $\geq 1:16$ was considered to be a high-titre positive. We classified household size as ≤ 5 or >5 residents, 5 householders being the national average according to the most recent census [17]. For each community, we used village level estimates of treatment coverage obtained during our previous six month follow

up survey [10]. We classified village level coverage as low (<80%), high (80-90%) or very high (>90%). We calculated the prevalence of latent and active yaws in older children (aged 5-14 years) and the sero-prevalence of exposure to yaws in younger children (aged 1-4 years). Logistic regression was used to estimate unadjusted and adjusted odds ratios (ORs) for factors associated with both TPPA- and dual-seropositivity. Robust standard errors were used to calculate all confidence intervals (CIs) and p-values, to account for village-level clustering. All analyses were performed using Stata 13.1 (Statacorp, Texas).

Sample size

In our previous follow-up survey, the seroprevalence of active yaws was 3.6%. We calculated that a sample size of 738 was need to measure a seroprevalence of 4% with a degree of absolute precision of 3%, assuming a design effect of 4.5 (estimated using data from the baseline survey) in children aged 5-14 years. Based on previous data we hypothesized that the seroprevalence amongst children aged 1-4 years would be approximately 1% if transmission had been interrupted following the earlier community mass treatment and 5% if transmission had not been interrupted. We calculated that a sample size of 432 children aged 1-4 years was required to detect a prevalence of 1% with a precision of 2% and a design effect of 4.

Ethical Approval

Written informed consent was obtained from each participating child's parent or guardian by a member of staff fluent in the local dialect. Assent was obtained from children. Ethical

approval for the study was granted by the ethics committees of the Solomon Islands MHMS, the CDC, and LSHTM.

Results

A total of 1,284 children were enrolled from 519 households in 10 communities.

811 children were aged 5-14 years (median 9 years), of whom 401 (49.4%) were male. A serum sample was collected from 770 children (94.9%). Thirty-one children (3.8%) declined collection of a serum sample but assented to collection of a dried-blood spot, whilst 10 children (1.2%) assented to examination but not to sample collection. 473 children aged 1-4 years (median 3 years) were also enrolled, of whom 248 (52.4%) were male. A dried blood spot was collected from 451 children (95.3%) in this age group. Seven children (1.5%) aged under 5 incorrectly had a serum sample (rather than a DBS) collected. No dried blood spot was collected from 15 children aged under 5 (3.2%).

Four hundred and thirty seven children (34.0%) had at least one skin lesion. 14 children (1.1%) had lesions considered to be clinically consistent with yaws. The most common non-yaws lesions were scabies and impetigo, as previously reported (A Steer, Personal Communication). As in our initial 6 month follow-up survey, no individual with a skin lesion consistent with yaws had dual-positive serology. Swabs were obtained from nine yaws-like lesions. Swabs could not be obtained from the other three lesions as they were dry/crusted. Of these lesion samples, all were negative on RT-PCR for *T.p* subsp *pertenue* and two were positive for *H. ducreyi*.

Amongst 5-14 year-old children, 223 had a positive TPPA (27.5%, 95% CI 13.6-47.7%). The TPPA seroprevalence amongst this age group did not differ significantly from either our pre-MDA survey or our initial follow-up survey (31.4% and 25.0% respectively, $p > 0.05$ for both comparisons). In both the crude and adjusted analyses, only age was associated with TPPA positivity (Table 2). Thirty-five children had dual positive serology (4.3%, 95% CI 2.1-8.7%), of whom 8 had high-titre positive serology. This did not differ significantly from our initial post-MDA follow-up survey (4.3% vs 3.5%, $p = 0.43$) but remained significantly lower than our initial pre-MDA survey (4.3% vs 21.7%, $p < 0.0001$). The level of MDA coverage was strongly associated with dual-seropositivity ($p = 0.005$). Compared to an MDA coverage above 90%, a village level coverage below 80% was strongly associated with an increased risk of dual-seropositivity (aOR 6.95, 95% CI 1.2-38.3, $p = 0.03$) (Table 3). Coverage of between 80-90% was also associated with an increased risk of dual-seropositivity compared to coverage $>90\%$, but this difference was not statistically significant (aOR 3.99, 95% CI 0.66-24.0, $p = 0.12$).

Amongst children aged 1-4 years, 16 had a positive TPPA (3.5%, 95% CI 1.6-7.1%) including two children aged less than two years (0.9%, 95% CI 0.2 – 4.0%). This did not differ significantly from the seroprevalence in this age group that had been predicted based on our previous surveys (3.5% vs 5%, $p = 0.11$). In villages where very high ($>90\%$) mass treatment coverage was achieved, there were no young children with a positive TPPA, compared to 3.8% and 4.4% TPPA positivity in villages with low ($<80\%$) and high (80-90%) coverage of mass treatment, respectively, although these differences were not statistically significant ($p = 0.08$ and 0.06 respectively). Age was the only variable significantly associated with TPPA seropositivity amongst children aged 1-4 years (Table 4).

Discussion

We have previously demonstrated that a single round of mass treatment with azithromycin at 20mg/kg, given for the purposes of trachoma control, has a significant impact on the prevalence of yaws-like skin lesions and sero-positivity for yaws [10]. In this study we have shown that the benefit appears to extend to 18 months without any further intervention. The WHO yaws eradication strategy [4] suggests that, following initial mass treatment, follow-up surveys with targeted treatment of cases and contacts should take place every 3-6 months. This requirement for at least biannual intervention is a potential barrier to integrating yaws eradication efforts with other NTD control programmes, including trachoma, where annual mass treatment is recommended. The lack of a significant change in seroprevalence from 6 to 18 months after mass treatment might suggest that interventions could be spaced at yearly intervals without a significant loss of impact. Modelling studies exploring these questions are under way, and initial results suggest that such decisions are highly dependent on the basic reproductive number (R_0) of yaws. Where R_0 is low (<1.45) then annually spaced treatment may be sufficient to achieve interruption of transmission (M Marks – Manuscript submitted).

Of particular interest, the village-level treatment coverage was the strongest risk factor for dual-seropositivity 18 months after mass treatment. When the coverage was below 80%, the risk for seropositivity was more than 6 times greater compared to villages where coverage of greater than 90% was achieved. Consistent with these findings in older children, we noted that in villages where very high ($>90\%$) coverage was achieved there were no seropositive children aged 1-4 years, which may indicate that transmission was interrupted in these communities. Ongoing presumed seroconversion, including in children aged 2 years or less,

was documented, however, in villages with coverage below 90%. We cannot exclude the possibility that positive serology in children aged 1-4 years was due to mother-to-child transmission of syphilis. However, in the context of ongoing transmission of yaws amongst older children in these communities, we consider it likely that seropositivity amongst children aged 1-4 years does reflect ongoing transmission of yaws in this age group. These two findings emphasize the need for extremely high coverage during mass treatment. Given the difficulties that can be faced in achieving such high coverage at a programmatic level [18], innovative strategies should be considered to augment yaws eradication efforts. This might include additional school-based mass treatment alongside community-based approaches, or use of multiple rounds of mass treatment before transitioning to the total targeted treatment phase of the yaws eradication strategy [4].

Our data add to the literature supporting a lower dose of azithromycin (20mg/kg max 1g vs 30mg/kg max 2g) for the treatment of yaws. If a lower dose is proven effective, this may help achieve programmatic synergies, especially in the Pacific where several countries have a high prevalence of yaws, and trachoma is also endemic. A WHO sponsored trial (NCT02344628) is being undertaken in Ghana and Papua New Guinea to formally compare the efficacy of these two doses in both active and latent yaws, and it is hoped that this study will provide a definitive answer on the efficacy of the lower dose of azithromycin.

The major limitation of our study is the observational design. A study of mass treatment in Papua New Guinea found only 44 serologically confirmed active cases in a total population of 13,166 individuals seen at six months follow-up. Given the smaller sample size of this study,

we may have been underpowered to detect rare active cases following the initial round of mass treatment.

Our data show that the impact of a single round of mass treatment on yaws prevalence is profound, and appears to last for at least 18 months following mass treatment, even in the absence of further interventions. We have demonstrated that mass treatment coverage above 90% is associated with significantly better outcomes than lower coverage. Strategies to improve coverage at all stages of yaws eradication efforts should be investigated.

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Table 1: Demographics of study subjects

Number of Children		1284
Number of Households		519
Household Size		6 (4-8)
[Number of residents]		
(Median, IQR)		
Age [years] (Median, IQR)		6 (3-10)
Village Level azithromycin Coverage	Low (<80%)	536 (41.7%)
	High (80-90%)	501 (39.0%)
	Very High (>90%)	247 (19.2%)

Table 2: Risk factors for TPPA positivity amongst children aged 5-14 years

Variable	Indicative Prevalence Data	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio[#] (95% CI)	p-value
Age [years]	5-9: 19%	1.22 * (1.13-1.32)	1.22 (1.13-1.32)	<0.001
	10-14: 38.5%			
Male	Male: 28.4%	1.09 (0.77-1.57)	1.19 (0.87-1.63)	0.24
	Female: 26.6%			
Household Size [number of residents]	≤5: 30.21%	1		
	>5: 25.6%	0.8 (0.48-1.31)	0.75 (0.46-1.23)	0.23
Village Level azithromycin coverage[¶]	Low Coverage (<80%): 24.1%	1.13 (0.23-5.48)	1.28 (0.26-6.39)	0.73
	High Coverage (80-90%): 33.4%	1.83 (0.34-10.09)	1.96 (0.31-12.56)	0.44
	Very High Coverage (>90%): 21.9%	1		

* Increased odds associated with each one year increase in age

Adjusted for age and gender

¶ Compared to very high coverage (>90%)

Table 3: Risk factors for dual-seropositivity amongst children aged 5-14 years

Variable	Indicative Prevalence Data	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio [#] (95% CI)	p-value
Age [years]	5-9: 3.3%	1.18* (1.05-1.34)	1.19 (1.08-1.35)	0.009
	10-14: 5.7%			
Male	Male: 3.2%	1.77 (0.85-3.71)	1.91 (0.93-3.94)	0.07
	Female: 5.5%			
Household Size [number of residents]	≤5: 5.7%	1		
	>5: 3.3%	0.57 (0.14-2.32)	0.53 (0.13-2.14)	0.33
Village Level azithromycin coverage[¶]	Low Coverage (<80%): 6.2%	5.5 (0.97-31.22)	6.95 (1.27-38.3)	0.03
	High Coverage (80-90%): 4.1%	3.6 (0.58-21.67)	3.99 (0.66-24.0)	0.12
	Very High Coverage (>90%): 1.2%	1		

* Increased odds associated with each one year increase in age

Adjusted for age and gender

¶ Compared to very high coverage (>90%)

Table 4: Risk factors for TPPA positivity amongst children aged 1-4

Variable	Indicative Prevalence Data	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio[#] (95% CI)	p-value
Age [years]	1-2: 0.87%	1		
	3-4: 5.8%	2.21* (1.55-3.15)	2.23 (1.51-3.29)	0.001
Male	Male: 4.0%	1.53 (0.58-4.06)	1.64 (0.56-4.82)	0.33
	Female: 2.7%	1		
Household Size [number of residents]	≤5: 4.0%	0.72 (0.25-2.08)	0.78 (0.25-2.35)	0.62
	>5: 2.9%	1		

* Increased odds associated with each one year increase in age

Adjusted for age and gender

Supporting information legends

Checklist S1: Strobe Checklist

File S2: Supplementary Data File

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of cross-sectional studies

Section/Topic	Item #	Recommendation	Reported on page of thesis #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	87-88
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	89-91
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	92-93
Objectives	3	State specific objectives, including any pre-specified hypotheses	92-93
Methods			
Study design	4	Present key elements of study design early in the paper	94
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	94
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	94-95
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	95-96
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment	95-96

		(measurement). Describe comparability of assessment methods if there is more than one group	
Bias	9	Describe any efforts to address potential sources of bias	95-96
Study size	10	Explain how the study size was arrived at	95-96
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	95-96
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	95-96
		(b) Describe any methods used to examine subgroups and interactions	N/A
		(c) Explain how missing data were addressed	N/A
		(d) If applicable, describe analytical methods taking account of sampling strategy	95-96
		(e) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—e.g. numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed	97
		(b) Give reasons for non-participation at each stage	97-98
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (e.g. demographic, clinical, social) and information on exposures and potential	97-98+ Table 1

		confounders	
		(b) Indicate number of participants with missing data for each variable of interest	97-98
Outcome data	15*	Report numbers of outcome events or summary measures	97-98
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included	97-98 and Table 2 & 3 & 4
		(b) Report category boundaries when continuous variables were categorized	97-98 and Table 2 & 3 & 4
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarize key results with reference to study objectives	99
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	99-100
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	99-100

Generalizability	21	Discuss the generalizability (external validity) of the study results	99-100
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	102

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

Chapter 5: Validation of a rapid diagnostic serological test for yaws



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RESEARCH PAPER COVER SHEET

PLEASE NOTE THAT A COVER SHEET MUST BE COMPLETED FOR EACH RESEARCH PAPER INCLUDED IN A THESIS.

SECTION A – Student Details

Student	MICHAEL MARLEY
Principal Supervisor	DAVID MABEY
Thesis Title	EPIDEMIOLOGY OF YAWS IN THE SOLIDARITY ISLANDS AND THE IMPACT OF A TREATMENT CONTROL PROGRAMME

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?	PLoS NEGLECTED TROPICAL DISEASES		
When was the work published?	2014		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	N/A		
Have you retained the copyright for the work?*	OPEN ACCESS CC LICENCE	Was the work subject to academic peer review?	YES

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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	
Stage of publication	

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I PERFORMED THE LAB WORK FOR THE GOLD STANDARD TEST, ANALYSED THE DATA, WROTE THE MANUSCRIPT AND RESPONDED TO THE PUBLISHER REVIEW COMMENTS
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Student Signature:

Date: 18/4/2016

Supervisor Signature:

Date: 18/4/16



Evaluation of a Rapid Diagnostic Test for Yaws Infection in a Community Surveillance Setting

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Abstract

Yaws is a non-venereal treponemal infection caused by *Treponema pallidum* ssp. *pertenue*. The WHO has launched a worldwide control programme, which aims to eradicate yaws by 2020. The development of a rapid diagnostic test (RDT) for serological diagnosis in the isolated communities affected by yaws is a key requirement for the successful implementation of the WHO strategy. We conducted a study to evaluate the utility of the DPP test in screening for yaws, utilizing samples collected as part of a community prevalence survey conducted in the Solomon Islands. 415 serum samples were tested using both traditional syphilis serology (TPPA and quantitative RPR) and the Chembio DPP Syphilis Screen and Confirm RDT. We calculated the sensitivity and specificity of the RDT as compared to gold standard serology. The sensitivity of the RDT against TPPA was 58.5% and the specificity was 97.6%. The sensitivity of the RDT against RPR was 41.7% and the specificity was 95.2%. The sensitivity of the DPP was strongly related to the RPR titre with a sensitivity of 92.0% for an RPR titre of >1/16. Wider access to DPP testing would improve our understanding of worldwide yaws case reporting and the test may play a key role in assessing patients presenting with yaws like lesions in a post-mass drug administration (MDA) setting.

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Data Availability: The authors confirm that, for approved reasons, some access restrictions apply to the data underlying the findings. Data concerning the results of the matched DPP and TPPA/RPR assays (which constitute the majority of the data analysed and presented in the paper) are available to all researchers as a supporting information file. The linked demographic, GPS and clinical data are subject to a data-sharing agreement mandated by the Solomon Islands Ethics Review Board and signed by the Solomon Islands Ministry of Health, LSHTM and Sightsavers, who helped to fund this research through the Global Trachoma Mapping Project grant from the Department for International Development of the United Kingdom. Making these data available without restriction would breach the terms of that agreement. Therefore, these data can be accessed through the Secretariat of the National Health Research Ethics Committee of the Solomon Islands by contacting Dr Andrew Darcy (walelumandrewd@gmail.com).

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Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Yaws is a non-venereal treponemal infection caused by *Treponema pallidum* ssp. *pertenue* (*T. pertenue*) [1] which is currently thought to be endemic in fourteen countries [2]. The emergence of azithromycin as an effective oral agent in the treatment of yaws [3] has prompted renewed calls for a coordinated worldwide programme to eradicate the disease by 2020 [4].

Despite this optimism there are significant barriers still to be overcome. The differential diagnosis of yaws can be broad [5] and serological testing is necessary to help establish a diagnosis. As with syphilis, latent infection occurs, and it is recognized that for every clinical case there may be 5–6 individuals with serological evidence of infection but no clinical manifestations [6,7]. Failure to adequately treat latent cases was one of the reasons for the failure of previous attempts to eliminate yaws [8]. Detection and treatment of these latent cases will be extremely important if the WHO eradication programme is to achieve its target.

Traditional syphilis serology includes a treponemal specific test, such as the *Treponema pallidum* particle agglutination assay (TPPA) or the fluorescent treponemal antibody test, and a non-treponemal test, such as the Rapid Plasma Reagin (RPR) or Venereal Disease Research Laboratory (VDRL) assays. The former tests are highly specific but normally remain positive for life following infection. The latter tests are non-specific but the RPR titre more accurately reflects disease activity and falls following successful treatment. Low-titre false-positive RPRs may occur in a number of conditions including acute viral infections, malaria and connective tissue diseases. Testing therefore requires combined treponemal and non-treponemal assays to give a more accurate diagnostic result.

The development of a rapid diagnostic test (RDT) that can be used to improve access to serological diagnosis in the isolated communities affected by yaws has been highlighted as a major research question [9] to be addressed. RDTs allow wider access to diagnostic testing in remote communities where laboratory

Author Summary

Yaws is a bacterial infection closely related to syphilis. The WHO has launched a worldwide campaign to eradicate yaws by 2020. If this goal is to be achieved, programme managers and clinical staff will need access to a rapid diagnostic test (RDT) for yaws that can be used in the remote communities where the disease is found. In this study, we present data evaluating one possible RDT for yaws as part of a community survey in the Solomon Islands. The test performed reasonably well—there were some false negatives but few false positives. The performance of the test was best in individuals with more active disease suggesting the test may be most appropriately used for confirming clinically diagnosed cases. These findings should prompt consideration of the use of this RDT as part of worldwide yaws control efforts.

facilities are not available, with results available at the point of care to inform clinical decision making. As yaws is serologically indistinguishable from syphilis [10], the recent development of syphilis RDTs with high sensitivity and specificity [11] prompts evaluation of their use for the diagnosis of endemic treponemal diseases.

The required role(s) of an RDT may vary depending on the progress of the eradication programme in a given country. The test may have utility to confirm the diagnosis in patients presenting with skin lesions, to detect ongoing transmission of infection after mass drug administration has been conducted, or to conduct community surveillance in areas previously known to be endemic. The target product profiles of the RDTs required in each of these settings are likely to vary.

The Dual Path Platform (DPP) Syphilis Screen and Confirm (Chembio, Medford, NY, USA) provides both a “treponemal” result (analogous to a *Treponema Pallidum* Particle Agglutination (TPPA) assay (T1 line)) and a “non-treponemal” result (analogous to a qualitative Rapid Plasma Reagin (RPR) assay (T2 line)) [12]. We conducted a study to evaluate the utility of the DPP test in screening for yaws in the general population, utilizing samples collected as part of a community prevalence survey for yaws and trachoma conducted in the Solomon Islands.

Methods

Participant Recruitment

This study was embedded in a larger study investigating the epidemiology of yaws in the Solomon Islands. Briefly, we undertook a survey in Western and Choiseul provinces of the Solomon Islands in September and October 2013. Twenty-five clusters were randomly selected in each province. In each cluster, thirty households were visited, and children aged five to fourteen were enrolled in the study. The study team collected information on yaws symptoms, signs and treatment history. Venepuncture was performed and a serum sample was collected from all participants. Sera were kept on wet ice (4°C) in the field and transferred within 5 days of collection to the National Referral Hospital, Honiara, where they were frozen. Samples were shipped on dry-ice to the London School of Hygiene & Tropical Medicine (LSHTM).

Sample Size

We assumed that the prevalence of yaws sero-positivity by the gold standard assay would be 30%. We therefore calculated that a sample size of 415 was required to be 80% confident that the true

Table 1. Participant characteristics.

Number of children		415
Gender: Male(%)	216	(52.1%)
Age (yrs): Median (IQR)	9	(7–11)
Clinical signs of primary yaws	12	(2.9%)
Clinical signs of secondary yaws	7	(1.7%)
Clinical signs of healed yaws	34	(8.2%)
Recent treatment for yaws	54	(13.0%)

doi:10.1371/journal.pntd.0003156.t001

sensitivity of the DPP, compared to the gold standard, was 85% or greater [13]. For the purposes of this study, simple random sampling was used to select samples to undergo parallel testing with both the DPP kit and traditional serology.

Laboratory Testing

Sera were tested using both the TPPA (Mast Diagnostics, Merseyside UK) and a quantitative RPR (Deben Diagnostics, Sheffield UK) at LSHTM by an operator masked to clinical findings. A second operator, masked to clinical findings and gold standard serology, tested samples using the DPP test kit. Samples for which the control line did not appear were repeated. The manufacturer’s instructions were followed for all test kits.

Statistical Analysis

The sensitivity, specificity, positive and negative predictive values of the DPP test kit were calculated using traditional serology as the gold standard. The DPP-T1 line was assessed against TPPA and the DPP-T2 line was assessed against RPR. Secondary analyses estimated these performance characteristics by RPR titre and presence or absence of clinical signs of yaws. Confidence intervals were calculated using robust standard errors to allow for clustering. Findings are reported in line with the STARD checklist for studies of diagnostic accuracy [14].

Ethics

Written, informed consent was obtained from the head of each household, who was the parent or guardian of children enrolled in



Figure 1. Clinical lesions of yaws. a) Primary yaws ulcer. b) Healed yaws lesion. Images credit: Michael Marks. doi:10.1371/journal.pntd.0003156.g001

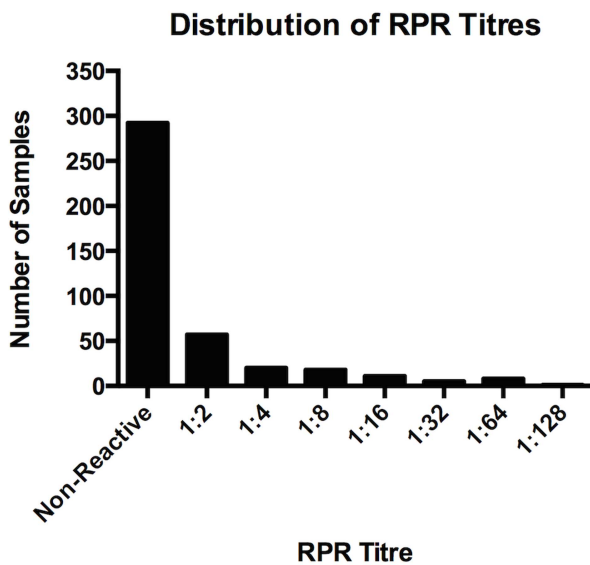


Figure 2. RPR titre distribution.
doi:10.1371/journal.pntd.0003156.g002

the study, and assent was obtained from all children. Ethical approval for the study was granted by the ethics committees of the Ministry of Health and Medical Services in the Solomon Islands, and LSHTM in the UK.

Results

Participant Characteristics

Four hundred and fifteen samples were randomly selected. The median age was 9, and 52.1% of participants were male. Individuals selected for this study did not differ significantly from the larger prevalence survey population with regards to demographic or clinical features (data not shown). Clinical findings consistent with active and healed yaws were found in 19 (4.7%) and 34 (8.2%) respectively of the 415 participants (Table 1 and Figure 1).

Laboratory Testing

123 (29.6%) individuals had a reactive TPPA. 120 (28.9%) individuals had a reactive RPR at any titre. By gold standard serology there were 18 individuals with a false positive RPR (defined as a positive RPR and negative TPPA). All false positive RPRs in our study had an RPR titre of 1:2. The overall prevalence of true RPR reactivity was therefore 102/415 (24.6%). The distribution of RPR titres in the study population is given in Figure 2.

79 individuals (19.0%) had a positive T1 (treponemal) line on the DPP kit. 64 (15.4%) individuals had a positive T2 (non-treponemal) line on the DPP kit. There were ten individuals with a positive T2 line but a negative T1 line. The overall prevalence of dual-positivity was therefore 54/415 (13.0%).

The sensitivity of the T1 line against TPPA was 58.5% and the specificity was 97.6%. The positive predictive value was 91.1% and the negative predictive 84.5%. The sensitivity of the T2 line against RPR was 41.7% and the specificity was 95.2%. The positive predictive value was 78.1% and the negative predictive value was 79.9%. The sensitivity of combined T1 and T2 against combined TPPA and RPR was 47.1% and the specificity was 98.1%. The sensitivity of the DPP was strongly related to the RPR titre (Tables 2 and 3)

In individuals with clinical signs of active yaws the sensitivity of the T1 line, compared to TPPA, was 81.8% and the specificity was 100%. The sensitivity of the T2, compared to RPR, was 55.6% and the specificity was 83.3%. A similar association was seen between T2 sensitivity and RPR titre as in the overall population (data not shown).

Discussion

In this study, we found that in a community surveillance setting, the sensitivity and specificity of the DPP rapid diagnostic test were markedly lower than in the only previous evaluation of the assay for use in clinically active yaws [15]. There was a strong association between the sensitivity of both the T2 line (against gold standard RPR) and RPR titre and also between the T1 line (against gold standard TPPA) and RPR titre. This finding might suggest that the apparent reduced sensitivity of the test reflects, at least in part, lower antibody titres in this population (where positive serology predominantly reflected asymptomatic latent cases) compared to populations in which the assay has previously been evaluated (where there were greater numbers of patients with active clinical disease), rather than a difference in test characteristics per se. Despite the reduced sensitivity and specificity the positive predictive values of the test remained relatively high, reflecting the high prevalence of treponemal infection in this endemic setting.

An association between the sensitivity of the T2 line and the RPR titre has previously been noted [12], but an association between RPR titre and T1 sensitivity has not been described before. It is possible that, in keeping with its lower pathogenicity compared to syphilis, yaws elicits less vigorous antibody production than does its venereal cousin, affecting both the non-specific (non-treponemal) and specific (treponemal) components of that response. ‘Attenuated yaws’ has previously been described in the Solomon Islands [16] with less florid clinical manifestations than noted elsewhere. Widespread use of antibiotics with treponemoidal activity has been postulated as one possible explanation for

Table 2. Test characteristics.

	Positive by Gold Standard	DPP Sensitivity	DPP Positive Predictive Value	DPP Specificity	DPP Negative Predictive Value
T1 (Treponemal Line) vs TPPA	123	58.5% (43.4–72.2%)	91.1%	97.6%(95.2–98.8%)	84.5%
T2 (Non-Treponemal Line) vs RPR	120	41.7% (28.0–56.8%)	95.2%	95.2%(92–95%)	79.9%
Dual Positive DPP (T1+T2) vs nDual Positive Gold-Standard Serology (TPPA+RPR)	102	47.1%(31.1–63.6%)	88.9%	98.1%(95.2–99.2%)	85.0%

doi:10.1371/journal.pntd.0003156.t002

Table 3. Test characteristics by RPR titre.

		Positive by Gold Standard	DPP Sensitivity	DPP Specificity
T2 (Non-Treponemal Line) vs RPR	RPR \geq 1/4	63	61.9%(44.8–76.5%)	92.8%(87.3–96.1%)
	RPR \geq 1/8	43	72.1%(51.4–86.3%)	91.1%(84.6–95.0%)
	RPR \geq 1/16	25	92.0% (66.6–98.5%)	89.4%(82.6–93.8%)
Dual Positive DPP (T1+T2) vs Dual Positive Gold-Standard Serology (TPPA+RPR)	RPR \geq 1/4	63	58.7%(41.3–74.2%)	95.2%(89.7–97.8%)
	RPR \geq 1/8	43	69.8%(48.3–85.1%)	93.5%(87.2–96.9%)
	RPR \geq 1/16	25	92.0%(66.6–98.5%)	92.1%(85.2–95.9%)

doi:10.1371/journal.pntd.0003156.t003

this postulated clinical entity. It is conceivable that this phenomenon could also contribute to the predominantly low-titre range of antibody responses seen in this study.

The setting in which this evaluation was carried out varies markedly from previous evaluations of the DPP test. In the largest published study, which evaluated the test in the diagnosis of syphilis, Yin and colleagues [11] evaluated the test in a population of 1,323 individuals presenting to a sexual health clinic in China, and found it to have a sensitivity of approximately 95% against TPPA and 86% against RPR. An association between RPR titre and sensitivity was also found in this study, although the performance of the test was better at titres of 1:4 and 1:8 than reported here.

Our study has a number of limitations. First, relatively few individuals tested had clinical evidence of active yaws, reflecting the community surveillance setting in which the test was evaluated. Whilst this limits our ability to comment on the value of the test for the purpose of case-confirmation, the aim of this study was to evaluate the DPP's use in screening whole communities at risk of yaws, and for this context we provide the first published data. Second, testing was performed in a central laboratory facility not in the field. Although the test can be performed rapidly (approximately 15–20 minutes per RDT), field-testing was not practical alongside the other activities being performed in our study, for which teams had to move house-to-house. Evaluations of the test elsewhere show that test performance is unaffected by whether venepuncture or finger-prick samples are used [11]. Further evaluations of the test in the field are warranted.

This study has implications for the use of the DPP RDT in yaws surveillance and control. Whilst the sensitivity of the test was lower than previously reported, the specificity remained high, and the negative and positive predictive values of the test were also high. Our data suggest that the DPP test can be used as part of a community surveillance strategy to identify individuals who are dually sero-positive with high-titre RPRs. These individuals are most likely to represent the major source of ongoing transmission.

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Identification of communities in which such individuals live is vital to allow adequate community targeted treatment to be undertaken [4]. The performance of the test in individuals with clinical evidence of yaws was better than in those without clinical evidence of disease, an association that is likely to be explained by the higher RPR titres found in individuals with clinically active disease. Because of inadequate access to point-of-care diagnostics, many national yaws surveillance systems only report clinically suspected cases. Wider access to DPP testing could allow a larger proportion of these cases to be evaluated serologically, which would both critically refine understanding of global yaws epidemiology as it evolves towards the eradication endpoint, but also provide a vital clinical aid in the post-MDA setting, where many conditions mimicking yaws will continue to present to health-care facilities. Further evaluation of the DPP in other yaws surveillance settings would be welcomed to provide the basis for guideline development.

Supporting Information

Checklist S1 STARD checklist. (DOC)

Dataset S1 Serology and DPP results. (XLS)

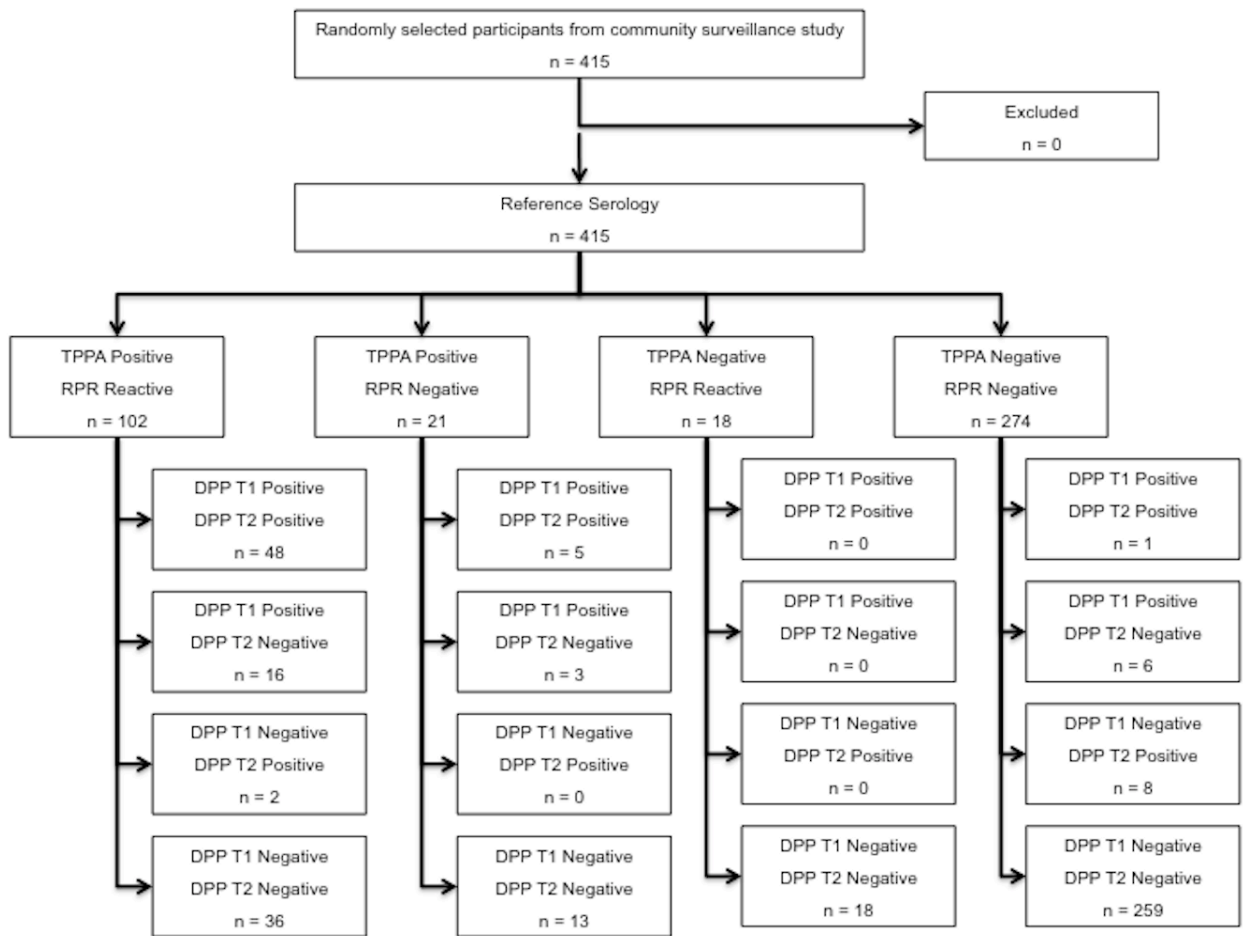
Figure S1 STARD flowchart. (TIFF)

Author Contributions

Conceived and designed the experiments: MM AG ZZ DM AWS. Performed the experiments: MM AG. Analyzed the data: MM AG VV ZZ CB DM AWS. Contributed reagents/materials/analysis tools: OS EP. Contributed to the writing of the manuscript: MM AG VV OS EP ZZ TD CB DM AWS. Designed and lead the fieldwork: MM VV OS EP ZZ TD AWS.

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Supplementary Figure 1: STARD flowchart



STARD checklist for reporting of studies of diagnostic accuracy

(version January 2003)

Section and Topic	Item #		On page #
TITLE/ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').	1
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	1-2
METHODS			
<i>Participants</i>	3	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	2
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	2
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.	2
	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	2
<i>Test methods</i>	7	The reference standard and its rationale.	2
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	2

	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	2
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.	2
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	2
<i>Statistical methods</i>	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	2
	13	Methods for calculating test reproducibility, if done.	N/A
RESULTS			
<i>Participants</i>	14	When study was performed, including beginning and end dates of recruitment.	3
	15	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).	3
	16	The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).	Supplementary Figure 1
<i>Test results</i>	17	Time-interval between the index tests and the reference standard, and any treatment administered in between.	N/A
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	3

	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	Table 2 and Table 3
	20	Any adverse events from performing the index tests or the reference standard.	N/A
<i>Estimates</i>	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	3 Table 2 and 3
	22	How indeterminate results, missing data and outliers of the index tests were handled.	3
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	N/A
	24	Estimates of test reproducibility, if done.	N/A
DISCUSSION	25	Discuss the clinical applicability of the study findings.	3-4

Chapter 6: Mathematical modeling to inform programmatic requirements for yaws eradication



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RESEARCH PAPER COVER SHEET

PLEASE NOTE THAT A COVER SHEET MUST BE COMPLETED FOR EACH RESEARCH PAPER INCLUDED IN A THESIS.

SECTION A – Student Details

Student	MICHAEL MARKS
Principal Supervisor	DAVID MABEY
Thesis Title	EPIDEMIOLOGY OF YAWS IN THE SOLOMON ISLANDS AND THE IMPACT OF A TRANSMISSION CONTROL PROGRAMME

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?	
When was the work published?	
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	
Have you retained the copyright for the work?*	Was the work subject to academic peer review?

**If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.*

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	EMERGING INFECTIOUS DISEASES
Please list the paper's authors in the intended authorship order:	MICHAEL MARKS, ORION MITOA, CHRISTOPHER FITZPATRICK, KINGSLEY ASIEDU ANTHONY SALOMON, DAVID MABEY, SEBASTIAN FUNK
Stage of publication	PEER REVIEW

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I DESIGNED THE TRANSMISSION MODEL, PARAMETERISED THE MODEL, WROTE THE INITIAL MODEL CODE, ANALYSED THE DATA AND WROTE THE MANUSCRIPT
--	--

Student Signature:

Date: 18/4/16

Supervisor Signature:

Date: 19/4/16

Mathematical Modelling of Programmatic Requirements for yaws eradication

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Key Words

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Abstract

Yaws is targeted for eradication by 2020. The mainstay of the eradication strategy is mass treatment followed by case finding. Modelling has been used to inform programmatic requirements for other NTDs and has the potential to provide insights into the yaws eradication strategy. We developed a model of yaws transmission varying the coverage and number of rounds of mass treatment. R_0 was estimated to be between 1.08 and 3.32. To have an 80% probability of achieving eradication eight rounds of treatment with 80% coverage were required at low estimates of R_0 . This rose to 95% at high estimates of R_0 . Extending the interval between treatments to 12 months significantly increased programmatic requirements at all estimates of R_0 . At high estimates of R_0 and when treatment rounds were spaced 12 months apart no combination of variables achieved eradication. Models should be used to guide the scale up of the eradication program.

Background

Yaws is a bacterial infection caused by *Treponema pallidum* subsp. *pertenue*¹. The disease predominantly affects children living in poor, remote communities and results in lesions of the skin, bone and cartilage. Previously, yaws was widespread throughout the tropics², but in the 20th century a series of control efforts based on mass treatment and case finding led by WHO is estimated to have reduced the burden of cases worldwide by up to 95%³. Despite these efforts, the disease has resurged in a number of countries in West and Central Africa, the Pacific and South-East Asia.

In 2012 it was demonstrated that a single dose of azithromycin was an effective treatment for yaws⁴. The availability of a well-tolerated oral agent has prompted WHO to develop a new eradication strategy, the 'Morges Strategy', based on community mass azithromycin treatment⁵. The strategy combines an initial round of total community treatment (TCT) followed by subsequent active case finding and total targeted treated (TTT) of newly identified cases and their contacts. Pilot studies have shown that community mass treatment with azithromycin is a highly effective strategy for reducing the community prevalence of yaws^{6,7}.

There are limited data to inform the optimum coverage and number of TCT or TTT rounds that are required to achieve elimination (interruption of transmission) of yaws at a local level to facilitate country-level elimination and ultimately global eradication. In India, a national yaws elimination campaign conducted between 1996 and 2004 resulted in significant reduction in the prevalence of yaws, sustained interruption of transmission and national elimination⁸. This programme consisted of six-monthly case finding surveys and treatment with parenteral penicillin. Whilst this approach did not include the initial mass treatment round now recommended as part of the Morges strategy, its success indicates that serial rounds of high coverage treatment may achieve local elimination.

A recent review of important research questions facing the global yaws eradication programme has highlighted the need for more accurate data to inform the optimum number and coverage of rounds of TCT and TTT that will be required to achieve yaws eradication⁹.

Mathematical modelling has been used to inform control efforts for a number of other Neglected Tropical Diseases (NTDs)¹⁰⁻¹² which are also managed using community mass treatment strategies, and such approaches could be of value for yaws eradication efforts. In particular, this approach may allow a comparison of the differential impact of alternative mass treatment strategies, which would be difficult to assess by empirical randomized controlled trials due to the size and cost of implementing large-scale cluster randomized studies.

Previous mathematical models for yaws¹³ have assessed the cost-effectiveness of yaws eradication, but have not directly addressed the feasibility of achieving this goal or the number of rounds of treatment that would be required. In this study, we aimed to determine whether the eradication of yaws is feasible based on the Morges strategy and, if it is, the number and coverage of mass treatment rounds needed to achieve the goal.

Methods

We developed a stochastic Markov model of community-level transmission of yaws (Figure 1). Upon infection, susceptible (S) individuals develop primary disease (I_1) at a rate proportional to the transmission rate β and the total number of infectious individuals ($I_1 + I_2$). Individuals with primary disease can further transition to secondary disease (I_2) at rate α , and both those with primary and secondary disease can transition to latent disease (L) at rate η . Lastly, those with latent disease can relapse back to I_2 at rate ρ . Infected individuals with primary or secondary disease are treated (and become susceptible again) at rate τ_i , and those with latent disease at rate τ_L . Unlike previous mathematical models of yaws¹³, tertiary yaws was not included in the model as these cases are believed not to contribute to transmission¹⁴. As individuals may be re-infected many times we did not consider individuals to obtain protective immunity following infection or treatment.

Although there is some evidence that there may be a non-human primate reservoir for yaws in Africa¹⁵ we did not include such infections in our model as there is currently no definitive evidence that the organism responsible for these infections is the same one that causes human yaws, or that zoonotic transmission occurs in the real world. We therefore considered the epidemiological significance of this possible reservoir as minimal when constructing our model.

Population Size

Estimates of the starting population for each compartment were derived from published population-based yaws prevalence studies^{6,16}. We modelled a discrete, closed population without addition or reduction via births or deaths.

Disease characteristic variable estimates (Table 1)

We estimated values for the rates of disease progression between different stages of yaws, including development of and relapse from latent yaws, using expert opinion, published data and estimates used in other models^{1,13,16}. We defined three transmission scenarios (low,

medium and high) using published age-specific treponemal seroprevalence data¹⁶, expert opinion, and values used in other yaws models¹³. Based on these data we generated initial estimates of R_0 of between 1.25 (low), 1.83 (medium) and 2.4 (high). These were converted to estimates of β , taking in to account the average duration of infection and the size of the population compartments in the model. The basic reproduction number R_0 taking account of the full structure of our model is 1.96 resulting in a mean R_0 of 1.45 (95% 1.01-2.14) for the low transmission settings, 1.95 (1.38-2.91) for medium and 2.47 for high (1.7-3.68) transmission settings. We included a variable to represent the likelihood of an individual receiving treatment for yaws in the absence of public health interventions based on published data¹⁶.

Mass drug administration variables estimates

We performed simulation experiments to estimate the impact of a yaws eradication intervention on disease transmission. In line with the Morges strategy⁵, we considered two programme components. In the first component, Total Community Treatment (TCT), all individuals were considered to have an equal chance of receiving treatment regardless of their infection status. In the second component of the intervention, Total Targeted Treatment (TTT), we considered that the coverage achieved amongst active and latent cases might differ. Intervention coverage was modelled independently for TCT, TTT pertaining to active cases, and TTT pertaining to latent cases over a range of plausible estimates (65% to 95% population coverage). Mass treatment compliance was simulated as a random, non-systematic process; i.e., each individual had the same chance of receiving treatment, with the likelihoods of any one individual receiving treatment being independent.

We varied the number of treatment rounds of both TCT (between 1-3) and TTT (between 0-6). Where one or more rounds of TTT were implemented these commenced following the final round of TCT. In line with the Morges strategy and real-world pilot implementations^{5,6}, rounds of mass treatment were spaced at six-monthly intervals.

We derived estimates of the efficacy of single dose treatment with azithromycin from randomized controlled trials of azithromycin for the treatment of yaws (Table 1).⁴ Following successful treatment, yaws lesions become non-infectious within 24 hours¹, and therefore we considered treatment to be immediately efficacious at the time of MDA, with individuals reverting to a susceptible state following treatment.

Implementing the model

The Markov model was run with an optimized tau-leap algorithm to approximate Gillespie's algorithm for exactly simulating stochastic systems^{17,18}.

We used the *adaptiveTau* package for R (the R Project for Statistical Computing)¹⁹. We performed repeated simulations across a range of disease transmission assumptions (β , $n=3$) and treatment assumptions, varying both the coverage and number of mass treatment rounds undertaken (Table 1).

For each combination of disease and intervention parameters we performed 1,000 simulation experiments. Within each combination we utilized latin-hypercube sampling using the *lhs* R package²⁰ to vary other disease specific variables (rate of progression and relapse, treatment in the absence of intervention) across the range of parameter estimates. We included an initial burn-in time of 50 months prior to the commencement of any yaws eradication intervention. The model then ran for a further 100 months.

Assessing outcomes

We recorded for each run of the model whether eradication was achieved. Eradication was defined as no cases of infectious or latent yaws at the end of the model run. The eradication probability was defined as the percentage of runs within each permutation of model characteristics where extinction was achieved. All analyses were performed using R version 3.2.2.

Results:

The model generated a total of 6,174 simulations of variable MDA strategies. As each strategy was implemented across a range ($n = 3$) of assumptions about the force of infection a total of 18,522 simulations were created.

The probability of achieving local interruption of transmission varied substantially across estimates of the force of infection and MDA characteristics.

At the lowest estimates of the force of infection, the minimum treatment thresholds required to have a transmission interruption probability of 80% or higher were a coverage of greater than 75% of all populations, and 8 or more rounds of treatment. Increasing the coverage to 85% reduced the total number of rounds required to 5. For comparison, when the gap between treatment rounds was extended from 6 to 12 months a total of 7 rounds of 85% coverage were required.

At medium estimates of the force of infection ($R_0 = 1.95$), the equivalent thresholds were 90% coverage and a total of 7 rounds of treatment. When the gap between rounds of treatment was increased to 12 months, no combination of treatment variables was predicted to have a transmission interruption probability of 80% or more.

At the highest estimates of the force of infection ($R_0 = 3.32$) a total of 8 rounds with 95% coverage were required for a greater than 80% likelihood of interrupting transmission. When the gap between rounds of treatment was increased to 12 months, no combination of treatment variables was predicted to have a probability of interrupting transmission of 80% or more (Figure 2).

It was considered plausible that, under field conditions, the coverage of latent cases would not exceed 70% in any given round of TTT, because such cases are not clinically apparent, and adequate coverage may not be achieved by treating the immediate contacts of clinical cases. At lower estimates of the force of infection, a total of three rounds of TCT with 85% coverage and three rounds of TTT (each with a coverage of active cases of 85% and latent

cases of 65%) was associated with a greater than 80% probability of interrupting transmission. If only 1 round of TCT was conducted, then coverage during TCT needed to be 90% and a total of 5 rounds of TTT (each with 90% coverage of active cases and 65% coverage of latent cases) were required. For medium estimates of the force of infection, a total of 8 rounds of treatment (3xTCT and 5xTTT) with a coverage of 90% were required. If only 1 round of TCT was undertaken, then 95% coverage was required, followed by 5 rounds of TTT with a 95% coverage of active cases and 70% coverage of latent cases. Under the highest estimate of the force of infection no combination of treatment variables was associated with a high probability of interrupting transmission.

Discussion:

Our study has demonstrated that, with implementation of the Morges strategy, interruption of transmission and, therefore, eradication of yaws is achievable, although not guaranteed. The probability of success would be strongly dependent on the transmission rate of yaws, and further studies to obtain better estimates of the basic reproduction number (R_0) in a range of countries where yaws is endemic would be of value to inform both improved models and programmatic planning. A minimum of eight rounds with coverage of at least 75% seems to be required for a high likelihood of achieving eradication, although this would prove inadequate at our highest estimates of possible values for R_0 . The predictions of our model are broadly in keeping with the real-world findings of the successful yaws elimination programme in India⁸ where 7 years of consecutive case finding and treatment (analogous to 14 rounds of TTT with 75% coverage) were conducted. In our model, the coverage achieved amongst latent cases during TTT has a profound impact on the likelihood of achieving eradication. When, in our model, coverage was limited to 70% or below, the required number of rounds of treatment to interrupt transmission increased considerably.

There are relatively few data available on the transmission rate of yaws. Even within endemic countries, the prevalence of yaws varies markedly. Studies in the Pacific have found a seroprevalence of anti-treponemal antibodies of more than 30% in a number of communities^{6,16} and a prevalence of clinical yaws of between 2.5-5% in pre-mass treatment communities. The prevalence of yaws is significantly lower in many endemic countries in West Africa²¹ but there are limited community based seroprevalence data available to inform our understanding of disease transmission there. We modeled a range of estimates of R_0 between 1.08 and 3.32 based on seroprevalence data and expert opinion. Given the significant influence of these estimates of R_0 on the likely outcome of community mass treatment, further studies to better understand disease transmission and how this varies within and between endemic communities would be of value. Ideally these studies would obtain community level age-specific seroprevalence data that could be used to calculate the force of infection. There is no perfect serological marker that can be used for this task. Traditional treponemal serology combines a treponemal test, which reflects lifetime

exposure but remains positive for life, with a non-treponemal test, the titre of which rises and falls following treatment. It is therefore not possible to use seroprevalence data to distinguish individuals who have been infected many times from those who have been infected once, and seroprevalence estimates are likely to under-estimate the true force of infection. For this study we calculated the force of infection relying on treponemal serology alone, which should provide a more accurate estimate of the force of infection than if we used dual positive serology. It remains, however, an imperfect measure.

Our model predicts that high coverage is required in all rounds of treatment to make yaws eradication feasible. Data from the previous WHO and UNICEF mass treatment campaign have highlighted the importance of achieving high coverage of latent cases²² and that treatment of active cases alone is insufficient to interrupt transmission. These factors were important considerations in the adoption as part of the Morges strategy of an initial round of total community treatment regardless of the prevalence of active disease in a community. Given the high coverage requirement, particularly of latent cases, and the relatively high fixed-costs of reaching endemic communities¹³ when compared to the relatively low costs of generic azithromycin, it may be preferable to conduct multiple rounds of Total Community Treatment prior to the switch to Total Targeted Treatment. Indeed such a recommendation would be in line with the original Morges Strategy²³, which recommended that additional rounds of TCT could be considered if the coverage achieved in the initial round of treatment was <90% or if access to endemic communities was difficult. A switch to multiple rounds of community mass treatment might also facilitate integration with other NTD MDA programmes in countries which are also frequently based on whole community mass treatment²⁴, although it should be noted that our model predicted a higher probability of achieving eradication with biannual treatment. Further studies to help determine the optimum strategy for achieving high coverage of latent cases during the TTT phase of eradication efforts should be considered – for example, to better understand the spatial epidemiology of latent yaws cases in relation to active cases in both pre and post-MDA settings or whether additional mass treatment rounds specifically targeting children might be beneficial.

Our study has a number of limitations. Most notably, we lack accurate estimates for a number of disease parameters. The disease parameters used in this study are broadly in line with those used by other models of yaws transmission¹³. We tested a range of coverage estimates for community mass treatment, but we did not factor in the possibility that some individuals may be systematically missed during mass treatment campaigns, a phenomenon that has been seen in control efforts for other NTDs²⁵. The current Morges strategy does not include adjunctive elements, such as WASH interventions, in addition to mass treatment, although some studies suggest that improved access to water and sanitation is associated with a decreased risk of yaws¹⁶. We did not include a secular trend in our model, and such a trend could be anticipated to further increase the likelihood of yaws eradication being achieved. Our model was designed to assess the feasibility of achieving yaws eradication in the near future, driven by the current WHO strategy, and in those conditions any effect of a secular trend could be expected to be minimal when compared to the significant impact of community mass treatment. Previous models have shown that secular trends are unlikely to significantly affect the cost-effectiveness of mass treatment¹³. It is important to note, however, that those models were based on an assumption of 90-99% coverage in a single TCT round and 100% coverage of index cases and their contacts in the TTT round.

Our study is the first to assess the theoretical achievability of worldwide yaws eradication, and represents an important milestone in reaching WHO's eradication target. We have defined programmatic thresholds that may need to be met to achieve yaws eradication, and identified key research questions to be addressed to inform both refinements of the model and worldwide roll-out of treatment strategies.

Table 1: Model Parameters

Parameter	Parameter Estimate	Comments
Epidemiological Parameters		
R_0	1.08 – 3.32	The average number of new cases occurring from a single index case in a fully susceptible population
Monthly probability of Progression from Primary to Secondary Disease without Treatment	2.78-5.56%	Untreated all individuals with primary disease either develop secondary or latent stage disease and this occurs over a period of 2-6 months.
Monthly probability of Progression from Infectious to Latent Disease without Treatment	13.9-27.8%	
Monthly probability of relapse from Latent Disease to Infectious stage without treatment	1-3%	
Population Parameters		
Susceptible at baseline	64%	Data derived from multiple pre-MDA surveys conducted in yaws endemic communities.
Primary yaws at baseline	1.5%	
Secondary yaws at baseline	1.5%	
Latent yaws at baseline	33%	
Mass Treatment Parameters		

TCT Coverage	65-95%	Coverage estimates were chosen to reflect the range achieved in real-world MDA programmes for other NTDs
TTT Coverage of Active Cases	65-95%	
TTT Coverage of Latent Cases	65-95%	
Number of Rounds of TCT	1-3	
Number of Rounds of TTT	0-5	

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Figure 1: Markov model of yaws transmission

Susceptible individuals (S) become infected at a rate dependent on both the transmission probability (β) and the number of individuals with infectious primary (I_1) and secondary (I_2) yaws. Without treatment individuals progress from primary disease to either latent yaws (L) or secondary yaws (I_2). Latent cases may relapse to generate further infectious secondary cases of yaws.

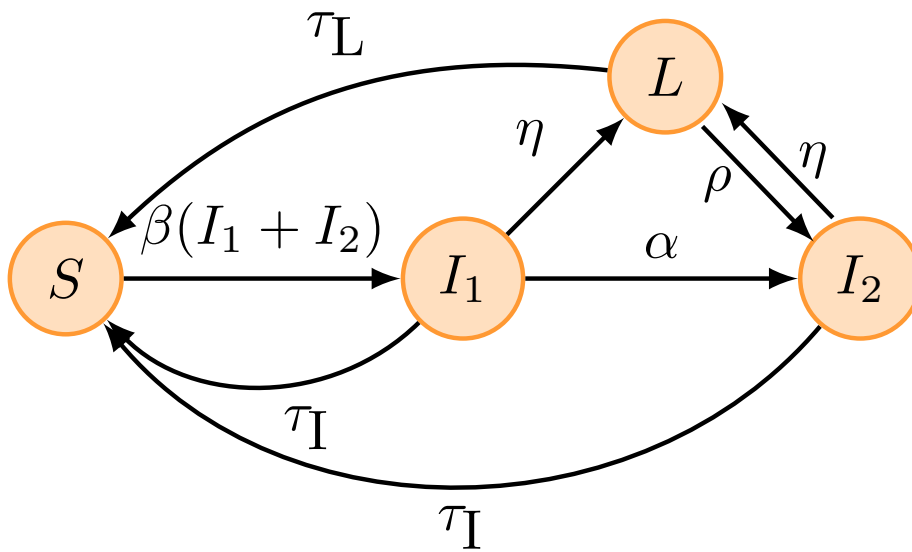
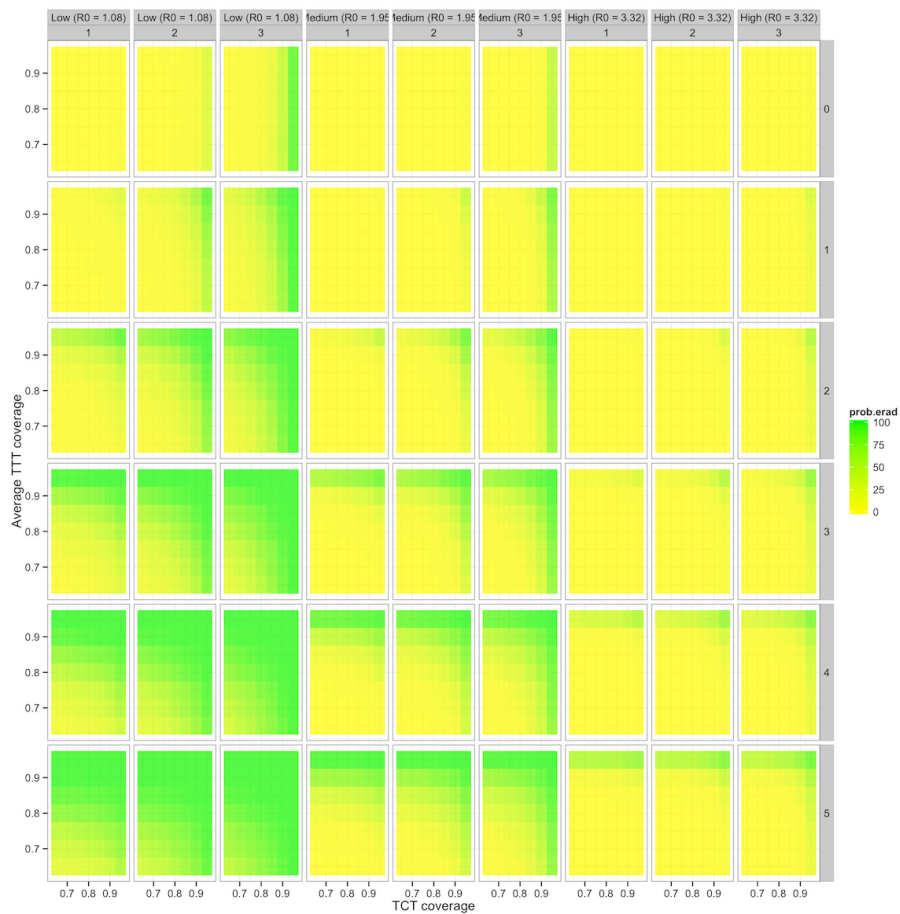


Figure 2: Probability of achieving eradication given different coverage estimates

The graphs show the predicted probability of achieving eradication given variations in the estimate of R_0 , the coverage of TCT (lower-X-axis) the number of rounds of TCT (upper-X-axis), the coverage of TTT (left-Y-axis), the number of rounds of TTT (right-Y-axis). For this graph we only show simulations where the coverage of latent cases is the same as the coverage of active cases during TTT. This may over-represent the true likelihood of achieving eradication, as the coverage of latent cases is likely to be lower than the coverage of active cases during TTT.



Chapter 7: Discussion

7. 1 Summary of research findings

This section provides a brief overview of the findings of the study in relation to the objectives of the thesis. For each objective, the Research Paper in which a detailed description of the study findings are reported, is highlighted.

Objective 1: Establish accurate data on epidemiology of yaws in Solomon Islands

(Research Paper - Chapter 3)

The pre-MDA study conducted in 2013 provided accurate prevalence estimates for both yaws infection and disease in the two provinces of the Solomon Islands where the study was conducted [1]. Almost one in three children (31.4%) had evidence of exposure in the form of a positive TPPA. The prevalence of both infection and disease was found to be higher in Western province than in Choiseul province, which is consistent with clinical case reporting through the national surveillance system. Significant clustering of infection was demonstrated at the sub-province level, a finding which has also been reported from other endemic settings [2]. The study was not able to identify clear drivers of geographical clustering and further studies to explore this would aid mapping and surveillance strategies.

Haemophilus ducreyi was identified as a common cause of skin ulcers in this study in individuals who were both seropositive and seronegative for yaws [3]. This finding, along with similar data from Papua New Guinea and Ghana [4,5], has been used to propose case definitions for yaws that vary at the different stages of an eradication effort [2]. In a pre-MDA setting, serology and clinical features are considered adequate, but in a post-MDA setting molecular tools are now recommended to supplement clinical and serological data.

Objective 2: Identify risk factors in pre-MDA setting for both yaws disease and infection

(Research Paper - Chapter 3)

The risk factor analysis conducted on the pre-MDA survey data demonstrated that the presence of other infected individuals at both the household and village level are the major factors associated with the risk of infection [1], which is consistent with the study's *a priori* hypothesis.

Consistent with other studies, an increased risk of infection in boys compared to girls was noted [6]. As yaws and syphilis are serologically indistinguishable, one explanation would be that these findings are due to early cases of sexually acquired syphilis amongst the males in the study. The association between gender and infection remained statistically significant even in the sub-group of children aged under 10 years, which makes this explanation less likely. An alternative hypothesis is that the additional risk in boys reflects increased traumatic damage to the skin, through rougher playing, which then serves as a portal of entry for infection by *T. p. pertenue*.

A possible protective effect from improved access to water and sanitation was noted, which might reflect improved skin integrity and a decreased risk of infection. This was a novel association, which had not previously been assessed in epidemiological studies of yaws and warrants further exploration. Further studies to better delineate the determinants of yaws epidemiology and guide appropriate survey design and health care interventions would be of value.

Objective 3: Assess impact of azithromycin MDA on yaws prevalence

(Research Paper – Chapter 4)

The two follow-up studies conducted during the PhD demonstrated a marked reduction in both clinical and serological evidence of yaws infection and disease. The seroprevalence of infection declined from 21.7% prior to MDA to 3.6% at six months and at eighteen months was 4.3%; no individual with both clinical and serological evidence of active yaws was found in either of the post-MDA surveys. These findings are consistent with the *a priori* hypothesis that treatment with 20mg/kg of azithromycin would effectively treat yaws and would result in a decreased prevalence of both active and latent yaws in the community. The results of this study are the first evidence to support the use of the lower dose of azithromycin for yaws, and provide important evidence for potential synergies between trachoma and yaws control programmes in the Pacific [7]. These studies also add to data from other settings that mass treatment for yaws is a highly effective intervention for the control of yaws [8].

Objective 4: Identify risk factors for treatment failure following MDA

(Research Paper – Chapter 4)

The six- and eighteen-month follow-up surveys identified non-receipt of treatment at the individual level and low coverage at the community level as the major factors associated with the risk of infection following mass treatment. At six months, individuals who had not received azithromycin were significantly more likely to have serological evidence of compared to those who had received treatment (aOR 3.9). In the eighteen month follow-up survey, the coverage achieved at the village level remained strongly associated with the risk of infection, with individuals in a village where coverage was below 80% having an adjusted odds ratio of 6 compared to individuals living in villages where a coverage of more than 90% was achieved. Taken together with the findings summarized below from the mathematical modelling studies, these data suggest that multiple high coverage (ideally > 90%) rounds of mass treatment will be required to interrupt transmission of yaws.

Objective 5: Evaluate diagnostic tests for use in yaws eradication programmes

(Research Paper – Chapter 5)

Current rapid syphilis tests have limitations as they only detect treponemal antibodies and can therefore not distinguish between past and current infection. In this study, the DPP-Syphilis Screen & Confirm RDT was shown to have high specificity (>95%) for both treponemal and non-treponemal antibodies [9]. The sensitivity of the test varied significantly with the reference RPR titre. For samples with an RPR titre of $\geq 1:16$, the sensitivity of the RDT was 92%, but this decreased to below 70% for samples with a RPR titre of $< 1:8$. These variations in sensitivity will need to be accounted for when considering the role of the RDT in yaws eradication campaigns. The greater sensitivity at higher titres suggests the RDT may be of use in mapping surveys and in active case finding during an intervention, but will be of less value in surveillance post-MDA, when lower titre disease will be more common and high sensitivity will be needed to ensure all cases are identified. Alternative strategies such as repeat testing of negative samples may be of value in increasing the sensitivity of the RDT. The finding that alternative organisms such as *Haemophilus ducreyi* may cause phenotypically similar ulcers highlights the fact that additional diagnostic tools such as PCR are likely to also play a central role in post-MDA surveillance [10,11]. Future evaluations should consider the optimum diagnostic strategy for each of the mapping, implementation and surveillance stages of yaws eradication.

Objective 6: Identify programmatic requirements for yaws eradication programmes

(Research Paper – Chapter 6)

Current recommendations about yaws eradication were developed on the basis of expert opinion and data from previous yaws eradication efforts conducted in the twentieth century [12–14]. Only one previous study had used mathematical modelling to address the question of yaws eradication, and the study was designed to measure the cost-effectiveness of an intervention, not its feasibility or programmatic requirements [15]. In this study, programmatic thresholds were identified to interrupt transmission under different epidemiological assumptions. At a coverage of 85%, the model predicted that five rounds of treatment would have a greater than 80% chance of achieving yaws eradication. The

coverage thresholds predicted are in-keeping with the marked effect of differential coverage demonstrated in the post-MDA follow-up surveys. Together, these data suggest that a strategy of administering multiple rounds of mass treatment before switching to active case finding may be more effective than use of a single round of mass treatment prior to that switch. Trials of this approach should be conducted. There is considerable uncertainty surrounding estimates of the R_0 of yaws and this should be a priority for further research studies.

7.2 Study limitations

Many of the findings in the study were derived from cross-sectional studies. This design has inherent limitations as it provides only a snapshot of the current epidemiology of the disease of interest at a specific point of time. There were limited data available to explore factors that might explain the marked clustering that was observed in the pre-MDA survey. Data were not collected on alternative foci of transmission such as schools or places of worship, or on other variables such as local climatic factors, which might be in part responsible for disease clustering. Fine-scale mapping capturing a wider range of environmental, infrastructure, and personal variables should be conducted to more accurately explore the determinants of clustering.

The assessment of the impact of mass treatment in this study was a before-and-after study design, which does not provide evidence that would be as robust for proving causality as evidence derived from a randomized trial. Whilst the findings from this study are consistent with other pilot implementations of azithromycin MDA for yaws, they do not provide direct evidence as to whether MDA with the higher dose of azithromycin would have been even more effective. A randomized controlled trial was not felt to be appropriate in the context of this study, where the MDA was being conducted by the Ministry of Health for a programmatic indication, but an RCT is being undertaken to provide definitive evidence on the comparative efficacy of the lower and higher doses of azithromycin (*Chapter 9 – Future Studies*).

Only one rapid diagnostic test was assessed in this study. The evaluation conducted in this study was conducted in a lab setting. The performance, quality control and use of rapid diagnostic tests can differ significantly in the field setting compared to in a lab. More detailed studies of the use of these test kits in 'real-world' settings are planned to allow a more detailed exploration of the optimal use of RDTs for yaws diagnostics (*Chapter 9 – Future Studies*). The role of alternative RDTs and diagnostic tests, including point of care nucleic acid amplification tests such as LAMP assays, warrants evaluation, as they may have a role in different stages of yaws eradication efforts.

The mathematical model only explored a limited number of treatment strategies, based on current WHO recommendations, and did not explore whether alternative approaches such as MDA targeted to children only, would be more efficient. The data were also derived from a limited set of data from the Pacific and use of other datasets to refine and validate the model should be considered.

7.3 Conclusions

Yaws is a significant public health problem in the Solomon Islands. In terms of the number of cases reported annually to WHO, the country is consistently amongst the three highest burden countries in the world [16]. The aim of this study was to establish accurate data on the epidemiology of yaws in the Solomon Islands, assess the impact of community mass treatment with azithromycin for trachoma on the prevalence of yaws, and generate data to support yaws eradication efforts.

Cross-sectional surveys were conducted before and after mass treatment to establish accurate estimates of the prevalence of infection and disease, and identify risk factors for disease before and after mass treatment.

In pre-mass treatment surveys exposure [1], latent infection and active disease were found to be extremely common. Household and village-level risk factors were the major factors associated with risk at an individual level. A new protective association between reported access to water and sanitation and risk of infection was noted. Significant clustering of disease was noted within endemic communities and future studies are required to understand the drivers of this clustering and their implications for both mapping and implementation of yaws eradication programmes. The pre-MDA studies demonstrated the weakness of clinical diagnosis for yaws. Many yaws-like lesions were found in seronegative individuals, and amongst seropositive cases, *Haemophilus ducreyi* was identified as an alternative aetiology in a large proportion of lesions [3]. The findings of these studies have significant implications for global yaws eradication efforts and highlight that point-of-care serological tests, augmented by molecular diagnostics, will be required.

This study provided the first data that a lower dose of azithromycin (20mg/kg) might be effective as a treatment for yaws. The study demonstrated a sustained reduction in prevalence following a single round of mass treatment, whilst also demonstrating that further interventions will be required to interrupt transmission. MDA coverage of 90% was associated with significantly lower risk of infection being present in a community at 18 months of follow-up. These findings were consistent with the findings of the mathematical modelling study and confirm the need for new approaches to increase the coverage achieved during treatment campaigns [17].

In summary, this PhD has addressed several key questions about the epidemiology of yaws. Even within endemic populations, the disease is highly focal. Integration of rapid diagnostic tests into routine surveillance may help improve data quality and guide yaws elimination efforts at a national level. Given the strong association between coverage of mass treatment and risk of infection, demonstrated in this study, new strategies to increase coverage are needed. Mathematical modelling may be of use in informing the design of these interventions.

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Chapter 8: Progress towards yaws eradication



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Yaws: towards the WHO eradication target

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In 2012 WHO declared a target to eradicate yaws by 2020. The cornerstone of this strategy is community mass treatment with azithromycin. Initial studies suggest this is a very effective tool that may be capable of interrupting transmission. Alongside this there has been progress in the development and validation of diagnostic tests for yaws. Several new challenges have also emerged, in particular, evidence that *Haemophilus ducreyi* can cause phenotypically similar ulcers in yaws endemic communities, and evidence for a possible non-human primate reservoir. The 2020 eradication target remains ambitious and more challenges should be expected on the journey.

Keywords: Eradication, Neglected Tropical Diseases, Yaws

In 2012 WHO launched a new strategy for the eradication of yaws¹ supported by World Health Assembly (WHA) resolution WHA 66.12, which called for global eradication of yaws by 2020. Yaws is predominantly a disease of children and, untreated, progresses to cause destructive lesions of the skin, soft tissues and bones. The disease is caused by *Treponema pallidum* subsp. *pertenue*, a spirochaete closely related to syphilis,² and is considered a neglected tropical disease (NTD). The disease can be treated effectively with a single-dose of either benzathine-penicillin or azithromycin. Mass treatment with azithromycin is the mainstay of the new WHO yaws eradication strategy.

Yaws has been the target of previous eradication efforts.³ Following WHA resolution 2.36, in 1949, a joint WHO/UNICEF eradication campaign treated more than 50 million individuals and is thought to have reduced the worldwide prevalence of yaws by 95%. Despite these successes, yaws eradication was not achieved. A number of factors contributed to this, including a failure to achieve adequate coverage of asymptomatic cases and inadequate surveillance following the completion of the initial phases of the eradication campaigns. The disease subsequently rebounded in a number of countries and despite a further WHA resolution in 1978 (WHA 31.58),³ and accompanying control efforts, the disease has continued to be a significant public health problem in a number of countries.

Globally yaws is known to be currently endemic in thirteen countries, predominantly in West Africa, South East Asia and the Pacific.⁴ For a further 76 countries, which have previously reported cases of yaws, there are no recent epidemiological data to guide eradication efforts. Two countries, India and

Ecuador, have reported local interruption of transmission of yaws following government led elimination efforts.

What are the prospects of achieving global yaws eradication? The feasibility of achieving eradication depends on biological, social and political factors.⁵

Biological factors

A fundamental requirement is the existence of a tool to interrupt transmission. A number of lines of evidence suggest that such a tool may exist for yaws. Previous eradication efforts using penicillin have shown that with extremely high coverage it is possible to interrupt transmission,³ a finding that has been confirmed by the recent experience of the Indian yaws elimination programme.

Will mass treatment with azithromycin be as effective? Initial pilot data are encouraging and suggest mass treatment with azithromycin has a significant impact on prevalence and transmission of yaws.⁶ A concern is the potential for emergence of resistance to azithromycin, now well documented in syphilis. Surveillance for resistance will be a vital component of yaws eradication efforts.

Adequate surveillance is needed at all stages of an eradication campaign. Where the clinical features of a disease are sensitive and specific, such as for guinea worm, clinical diagnosis alone may be sufficient. The clinical phenotype of early yaws is much less robust. In particular *Haemophilus ducreyi* has recently been shown to be a major cause of similar skin lesions in yaws endemic communities,⁷ in some cases accounting for more

cases than yaws. A rapid serological test has been validated for yaws but, as *H.ducreyi* has been found both in sero-positive and sero-negative individuals, serological diagnosis alone is unlikely to be sufficient. Access to serological diagnostics, and in the certification phase molecular diagnostics, will need to be scaled-up significantly if the eradication target is to be achieved.

A key requirement for disease eradication is the absence of an animal reservoir. An emerging body of evidence suggests there may be a non-human primate (NHP) reservoir for yaws. Serological and molecular evidence for a disease caused by *T.pallidum* has now been documented in NHPs across a range of countries in Africa, which are currently or formerly endemic for yaws.⁸ Alongside this ecological data, there is evidence that experimental infection of humans with NHPs strains is possible, and vice versa. Whether zoonotic transmission occurs in the wild remains an open but vital question.

Social and political factors

Social and political support is of fundamental importance for the success of any eradication campaign. The WHA resolution is an important step towards galvanising international political support for yaws eradication. As a disease predominantly of morbidity not mortality, yaws may not be perceived as a public health priority even in the countries where it is endemic. Maintaining political support is likely to become an increasing challenge as WHO scales up eradication efforts, global case numbers fall but the costs of on-going eradication efforts remain high.

Modelling work suggests that yaws eradication is cost-effective in the long term and it is hoped that this will help convince donors to support yaws eradication.⁹ These findings are based on a number of assumptions, which may not hold true. Firstly, the model assumed that transmission would be interrupted everywhere by a limited number of rounds of high coverage treatment (90–99% coverage), although there are limited empirical data to support this. Secondly, data from NTD programmes shows that such high coverage is rarely achieved in the real world.¹⁰ Finally, the model assumed that only surveillance would be required in all of the formerly endemic countries. If any of these countries were found to be currently endemic then the overall costs of yaws eradication may rise sharply.

Finally, many other NTD elimination/eradication programmes have benefitted from the creation of donation schemes to support drug and implementation costs. At present no such

donation programme exists for yaws and this represents a significant barrier to the scaling up of yaws eradication efforts.

Conclusions

There are four years remaining until the 2020 target for yaws eradication. At this point it seems unlikely that this target will be met. The first phases of yaws eradication have already revealed a number of unexpected findings and it seems likely that further obstacles will emerge on the road to eradication. Despite these obstacles, progress has been made in the development of new diagnostic tools and pilot studies demonstrating the effectiveness of azithromycin mass treatment. A sustained commitment from the WHO, countries and the academic community will be required if the goal of yaws eradication is to be achieved.

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Chapter 9: Future studies

The findings of this PhD and other studies conducted over the same time period are informing the design of future studies. As part of the PhD I have helped to draft the research priorities for achieving yaws eradication [1].

Further mapping studies are being planned in a number of yaws endemic countries where there are currently inadequate epidemiological data to guide programmatic decision-making. Data from these studies will feed in to refinements of mathematical models including spatial modelling and into the design of optimum sampling strategies for both pre and post-MDA yaws surveys. On-going work is exploring how integrated surveillance for multiple NTDs can be undertaken, either by use of shared sampling methodologies, or by testing a single sample, such as a dried blood spot, for multiple targets simultaneously [2]. The finding that other organisms may be responsible for yaws-like lesions in endemic communities has profound implications for surveillance [3]. More detailed molecular epidemiological studies are required to explore the relative importance of these organisms and understand their relevance within the context of yaws eradication efforts.

Emerging epidemiological data suggests that non-human primates may be a possible reservoir for yaws [4]. Whilst not relevant in the Pacific, this finding would have significant implications for global yaws eradication efforts. Collaborations are planned with groups with expertise in zoonotic infections in both Africa and Asia to explore the relevance of these findings to human yaws.

A number of future studies are planned to investigate how mass treatment for yaws can be optimized. These include studies of the optimum dose of azithromycin, alternative delivery strategies for MDA and whether MDA for yaws can be integrated with other NTD control programmes.

In line with our finding that a lower dose of azithromycin appears to be effective in the treatment of yaws, a formal randomized controlled non-inferiority study comparing the lower dose (20mg/kg) and higher dose (30mg/kg) of azithromycin in the treatment of both

active and latent yaws (NCT02344628) has been commenced. The study will recruit patients in both Ghana and Papua New Guinea, and aims to provide definitive data on the efficacy of the lower dose of azithromycin.

Informed by the findings from both the fieldwork and modelling studies of this PhD, a cluster-randomized study is being designed to assess different strategies to improve the effectiveness of yaws eradication strategies. The trial will compare a control arm of the standard WHO strategy with two alternative strategies; the first experimental arm will utilize multiple rounds of community mass drug administration prior to active case finding, whilst the second experimental arm will add school based mass drug administration to the initial community based mass drug administration. These studies will help inform more detailed mathematical models and guide scale up of yaws eradication efforts.

Co-administration is an attractive strategy to potentially increase coverage and efficiency of NTD MDA programmes. A lack of large field studies on the feasibility and safety of co-administration remains a barrier to its implementation. Working with colleagues at the University of Melbourne and the University of New South Wales, I am investigating the safety, feasibility and efficacy of co-administration of azithromycin and ivermectin as part of combined intervention for trachoma and scabies. A combined approach would be of particular value in the Pacific where scabies, yaws and trachoma are all endemic [5,6]. A further study of co-administration of azithromycin, albendazole and ivermectin is planned in Ethiopia. Taken together, these studies should form the basis for generating scientific consensus about co-administration.

An impact of mass drug administration on 'off-target' organisms has previously been demonstrated. In the setting of azithromycin MDA for trachoma an increase in resistant pneumococcus has been demonstrated in nasopharyngeal samples [7]. There are no current data on the impact of azithromycin MDA on other skin organisms. Both *Streptococcus pyogenes* and *Staphylococcus aureus* are common causes of skin disease in the tropics, particularly where scabies is endemic [6]. Mass drug administration with azithromycin might

have a beneficial impact in reducing the prevalence of impetigo but could also prove harmful if it results in an increase in resistant organisms. A study to assess the impact of azithromycin on common skin organisms using both culture and molecular based diagnostics is being planned and will be undertaken in Malaita province of the Solomon Islands.

Studies are planned to operationalize the use of the DPP-RDT in yaws endemic communities. These studies are designed both to design quality control measures that can be implemented in a resource poor setting and to use mixed methods to assess the acceptability of the test to both healthcare providers and patients.

The development of an integrated approach to Neglected Tropical Diseases is a major focus of future work. Consolidating vertical programmes is important to help support fragile health systems, maximise efficiency and realise crosscutting programmatic benefits. It is hoped that the work presented in this thesis and the future studies outlined above will contribute to making this a reality.

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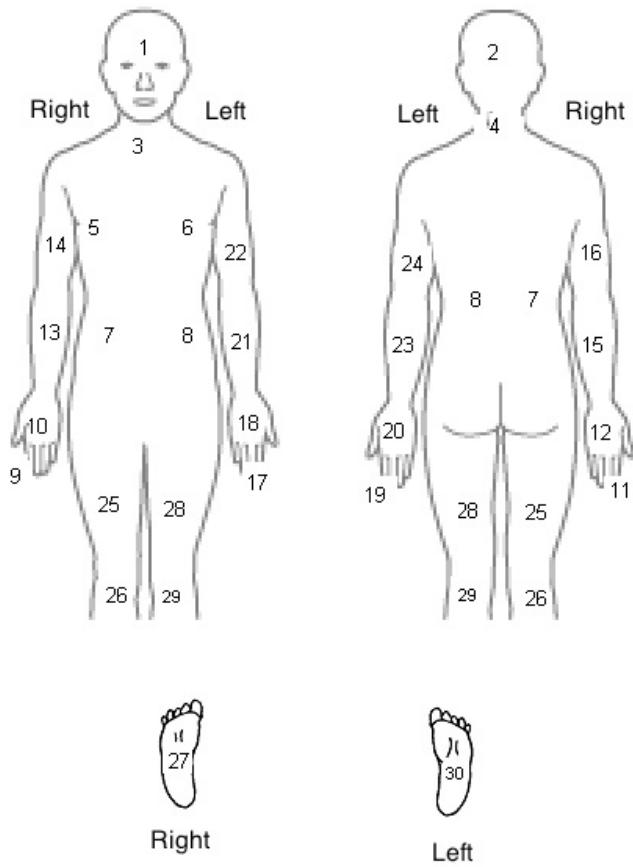
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Chapter 10: Appendices

Appendix 10.1: Skin lesion appearance coding

1. Papilloma: single or multiple yellow bumps on the skin.
2. Ulcer
3. Painful Ulcer
4. Painless Ulcer
5. Squamous Macule: Scaly, thickened or discoloured skin patches
6. Palmar/Plantar Lesion: holes, cracks, discolouration on soles of feet or palms of hands
7. Healed lesion: re-epithelized or healed lesion
8. Non-Yaws Lesion: Free Text Comment

Appendix 10.2: Skin lesion location coding



Appendix 10.3: Sampling, storage and transport of samples for yaws testing

Samples should be submitted for laboratory testing in accordance with the procedures for collection, storage and transportation described below.

Lesions

1. Use one or two sterile dacron- or cotton-tipped swabs to collect material from primary or secondary lesions.
2. Gently press and roll the swab along the lesion taking care to collect exude and skin.
3. Suspend one swab in a cyrotube containing 1ml transport medium (*AssayAssure* , <http://www.sierramolecular.com/products>). Press and roll the swab along the side of the tube to express the clinical material and excess fluid. **Discard the swab.**
4. Place the second swab onto the Indicating FTA Elute MicroCard (GE Healthcare) using 3 side-to-side motions, 90 degrees each way while pressing onto the card. Discard the swab and allow the card to air dry at room temperature completely (approximately 3 hours).

Venepuncture samples

1. Clean the skin with an alcohol swab and allow to dry.
2. Perform venepuncture using aseptic non-touch technique.
3. Collect 1 mL of blood in a serum tube (red top) for serology.
4. Discard the syringe in to an appropriate sharps container.

Dried-Blood Spots

1. Clean the finger to be pricked with an alcohol swab and allow to dry.
2. Prick the internal side of the finger using a sterile lancet. Discard the lancet into a sharp's container.

3. After pricking the finger, collect the blood directly onto each extension. Be sure the extensions are completely red on both sides (no white showing).
4. Place the labelled disk onto a pencil inserted into a styrofoam drying rack. Completely air-dry the disks at room temperature overnight if possible or for at least 2 hours. To avoid contamination, disks should not touch each other while drying.
5. When the disk has dried completely, place it in a small plastic bag with a desiccant sachet.

Conservation and transport of samples

Samples collected in to *AssayAssure* transport medium can be stored at room temperature for a maximum of two weeks but should ideally be stored in a refrigerator (4°C) or freezer (-20°C). Filter paper samples (dried blood samples and FTA Elute Microcards) should be stored in a sealed bag along with a desiccant sachet.

Frozen specimens should be packaged appropriately and shipped on dry ice to the laboratory for testing. If dry ice is not available, samples should be placed with frozen cold packs in a tightly sealed Styrofoam box. Filter paper cards can be shipped either frozen or at room temperature.

Appendix 10.4: LSHTM lab protocol

V1.0 (14/01/2014)

This standard operating procedure outlines the approach to testing of serum samples collected as part of the research project “Yaws in the Solomon Islands: impact of a national trachoma control programme on yaws clinical disease, seroprevalence and drug resistance.”

Samples will be tested with TPPA and RPR.

Initial testing will be

- TPPA
- RPR at ½ Dilution

Additional RPR dilutions will be completed on samples that are TPPA positive and RPR reactive at ½.

Sample Preparation

Samples are prepared to test in blocks of 94 specimens at a time.

Preparation of a Master Plates

- Samples are plated into a 96 deep well plate
- Spaces A1/B1 are left blank.
- Defrost serum sample
- Vortex the sample
- Pipette 160 microlitres into the well

TPPA Protocol

- Reconstitute sensitized and unsensitized TPPA particle kits with reconstitution solution – **30 minutes** before needed
- Put 100 microlitres of diluent in rows 1, 5 and 9
- Put 25 microlitres of diluent in row 2 to 4, 6 to 8 and 10 to 12.
- Add 25 microlitres of sample to wells 1, 5 and 9 and mix
- Transfer 25 microlitres from row 1 to 2, 2 to 3 and 3 to 4 mixing at each step
- Add 1 drop of 25 microlitres of unsensitized particles to wells 3, 7 and 11
- Add 1 drop of 25 microlitres of sensitized particles to wells 4, 8 and 12
- Cover the plate
- Gently shake the plate
- Cover for 2 hours
- Read result at 2 hours
- Repeat testing on any indeterminate samples

RPR Protocol

Initial Testing:

Each card has ten spaces. We will use 8 for testing samples and 2 for positive/negative controls. We will be testing $\frac{1}{2}$ dilutions.

1. For each sample
 - a. Prepare a $\frac{1}{2}$ dilution sample of total volume 50 microlitres
 - i. Pipette 25 microlitres of specimen in circle
 - ii. Pipette 25 microlitres of isotonic saline in circle
 - b. Add one drop of antigen to each circle
 - c. Spread within circle
2. Add Positive and Negative controls to appropriate spaces on the test card
3. Rotate test card for 8 minutes

4. Read result immediately
5. Repeat testing on any indeterminate samples

Quantitative

1. Prepare a plate containing dilutions of each sample across the range $\frac{1}{2}$ through to $\frac{1}{512}$
2. Test each dilution as per RPR protocol