Universidade Nova de Lisboa

Instituto de Higiene e Medicina Tropical

Refining the guidelines for the treatment and management of Cutaneous Ulcers through research in endemic populations of Papua New Guinea

Camila González-Beiras

TÍTULO PARA A OBTENÇÃO DO GRAU DE DOUTOR EM SAÚDE PÚBLICA GLOBAL

JUNHO 2018

Apoio financieiro da Fundação para a Ciência e a Tecnologia (FCT)

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Título para a obtenção do grau de doutor em Saúde Pública Global

List of publications

This thesis is based on seven papers listed below, which will be referred to throughout the text by their Roman numerals.

I. Mitjà, O., Marks, M., Konan, D., Ayelo, G., **Gonzalez-Beiras, C**., Boua, B., Houinei, W., Kobara, Y., Tabah, E., Nsiire, A., Obvala, D., Taleo, F., Djupuri, R., Zaixing, Z., Utzinger, J., Vestergaard, L., Bassat, Q. and Asiedu, K. (**2015**). Global epidemiology of yaws: a systematic review. *The Lancet Global Health*, 3(6), pp.e324-e331.

II. **González-Beiras, C**., Marks, M., Chen, C., Roberts, S. and Mitjà, O. (**2016**). Epidemiology of Haemophilus ducreyi Infections. *Emerging Infectious Diseases*, 22(1), pp.1-8.

III. **González-Beiras, C**., Kapa, A., Vall Mayans, M., Paru, R., Gavilán, S., Houinei, W., Bieb, S., Sanz, S., Martíns, R. and Mitjà, O. (**2017**). Single dose azithromycin for the treatment of Haemophilus ducreyi skin ulcers in Papua New Guinea. *Clinical Infectious Diseases*. 65(12) 2085-2090

IV. Mitjà O, **González-Beiras C**, Godornes C, Reman K, Houinei W, Abel H, Kapa A, Paru R, Bieb S, Wangi J, Sanz S, Asieu K, Lukehart S, Bassat Q. (**2017**) Effectiveness of single-dose azithromycin to treat latent yaws: a longitudinal comparative cohort study. *The Lancet Global Health*. .5(12) e1268–74

V. Grant, JC., * **González-Beiras, C*.,** Fortney, KR., Gangaiah, D., Humphreys, TL., Amick, KM., Mitjà, O., Abecasis, A., and. Spinola, SM. (**2018**) Multiple class I and class II Haemophilus ducreyi strains cause cutaneous ulcers in children on an endemic island. *Clinical Infectious Diseases* (In press). ***Co-first authors**

VI. Houinei, W., Godornes, C., Kapa, A., Knauf, S., Mooring, E., **González-Beiras, C**., Watup, R., Paru, R., Advent, P., Bieb, S., Sanz, S., Bassat, Q., Spinola, S., Lukehart, S. and Mitjà, O. (**2017**). Haemophilus ducreyi DNA is detectable on the skin of asymptomatic children, flies and fomites in villages of Papua New Guinea. *PLOS Neglected Tropical Diseases,* 11(5), p.e0004958.

VII. Oriol Mitjà, Charmie Godornes, Wendy Houinei, August Kapa, Raymond Paru, Haina Abel, **Camila González-Beiras**, Sivauk V. Bieb, James Wangi, Alyssa E. Barry, Kingsley Asiedu, Sergi Sanz, Quique Bassat, Sheila A. Lukehart. (**2018**). Re-emergence of yaws after single mass azithromycin treatment followed by targeted treatment: a longitudinal study. *The Lancet*. Published online February 7, 2018

To Professors C. Haddock and C. Darwin

for showing me the simplicity of life.

A los Profesores C. Haddock y C. Darwin

por mostrarme la simplicidad de la vida.

Acknowledgments

I'm writing this dissertation during my sixth rotation in Lihir island, two years after my first visit to Papua New Guinea. Having the chance to do my first work as a researcher in this remote island in the Melanesian has been the most exciting and enriching adventure I could have ever asked for, and I would like to thank and acknowledge all of those who have made this experience possible:

First of all, this work would not have been possible without the financial and logistical support of the Fundação para a Ciência e a Tecnologia, ISGlobal Barcelona Institute for Global Health and International SOS PNG.

My gratitude to the four portuguese schools (ENSP, IHMT, ISPUP and NOVA medical school) that put together this international PhD Global Public Health programme giving young researchers the chance to start their careers in Global Health.

All my gratitude to my advisors at my school, IHMT: Professor Rosario Martins, for believing in me and this project from the very beginning, and Professor Ana Abecasis, for the countless hours in our computers patiently teaching me skills I needed to complete this work. Furthermore, I'd like to thank the nine extraordinary women who walked this challenging years next to me: Maria, Ana A, Klara, Ana F, Hamida, Mariana, Ana T, Sanni, Anita: you have been the greatest colleagues and most loving friends.

I am specially indebted to the incredible Public Health team in Lihir: August, Kolmau, Rose, Paul, Ray,... It has been thanks to your patience and continuous support that I have learnt to walk the ways and customs of PNG. Tenkyu tru!. A specially loving acknowledgment to all the Lihirian children who have been part of this project: thank you for making toughest field days an absolute breeze with your laughter and smiles.

To Sergi, Pere and Ross. Working in Lihir is as wonderful as it can be challenging; this is a tough work place that poses a continuous mental, physical and emotional battle, the type of battle you can't overcome without loyal friends by your side.

Foremost, I am grateful to my mentor, Dr. Oriol Mitjà, for enlightening me the first glance of research, for your patience, motivation, enthusiasm and immense knowledge, for showing me the wonders of Papua New Guinea…thank you. I could not have imagined having a better advisor and mentor during this journey.

Finally, to my mum María, my dad Angel, and my siblings Carmen and Antón. You are the source of my strength. Everything good I am is a reflection of you.






Lihir Island, New Ireland Province

Papua New Guinea 2017-2018

Resumo (Português)

***Palavras-chave*:** *Úlcera Cutânea, Haemophilus ducreyi, Bouba, Treponema pallidum pertenue, Papua Nova Guiné.*

As úlceras cutâneas (UC), lesões cutâneas com perda das camadas superficiais da pele, são comumente encontradas em países de baixo rendimento da África e do Pacífico Sul. Em certas áreas remotas e rurais, como as ilhas da Papua Nova Guiné, a prevalência e a morbidade de UC são muito altas, representando, portanto, uma importante causa de sofrimento e estigmatização em crianças. Certas UC, se não forem tratadas, podem resultar em deformidades permanentes que afetam a vida das pessoas e que estão associadas a pobreza.

Embora vários agentes patogénicos possam causar UC, dois deles representam um grande problema de saúde pública nas ilhas do Pacífico Sul - bouba (Treponema pallidum pertenue) e *Haemophilus ducreyi*. Estes dois agentes patogénicos são a base desta tese e foram selecionados por variadas razões: em primeiro lugar, são as duas causas mais comuns de UC na região tropical do Pacífico Sul, onde os estudos para esta tese foram realizados. Em segundo lugar, estes são co endémicos, mas a sua interação como doenças infeciosas não foi estudada. Em terceiro lugar, por motivos epidemiológicos, clínicos e terapêuticos, as duas doenças são potencialmente tratáveis de forma integrada por meio do tratamento total da comunidade (TTC) com administração em massa de antibióticos (AMM) .

A bouba é classificada pela Organização Mundial da Saúde (OMS) como uma doença tropical negligenciada (DTN) que afeta principalmente crianças de 6 a 15 anos de idade em comunidades com falta de água e sem saneamento, levando à desfiguração e incapacidade crónica. No passado, a bouba foi sempre considerada a causa mais comum de UC, mas estudos de coorte recentes conduzidos na Papua Nova Guiné (PNG), Ilhas Salomão e Vanuatu identificaram o *H. ducreyi* como o agente etiológico que causa mais de metade de todas as UCs nos trópicos.

Em cenários endémicos de bouba rural, onde há ausência de diagnósticos moleculares, as UCs são classificadas com base nos resultados dos testes sorológicos da sífilis. Por exemplo, uma UC com um teste serológico positivo é classificada como bouba e tratada com azitromicina oral de dose única, enquanto uma UC com um resultado serológico negativo é classificada como úlceras não-bouba (por exemplo, *H. ducreyi* ou outros agentes patogénicos) e tratada com curso de 5-7 dias de diferentes antibióticos de amplo espectro. Esquemas de dosagem de múltiplos dias de tratamento e várias vezes por dia comprometem a adesão e satisfação do paciente.

Recentemente, um alarmante ressurgimento de bouba foi observado em vários países nos trópicos. O maior número de casos é relatado na Papua Nova Guiné, Ilhas Salomão e Gana. A OMS reagiu ao ressurgimento de bouba conduzindo campanhas de eliminação em áreas altamente endémicas usando tratamento empírico de UC com azitromicina oral de dose única. No entanto, se somente a bouba for tratada durante as campanhas, as comunidades podem ter a perceção de que a persistência de outros tipos de UC estará relacionada com um desfecho negativo da estratégia. A recente descoberta de que *H. ducreyi* é um dos principais agentes causadores de UC reforça a necessidade de maior investigação sobre o assunto.

É preciso explorar novas estratégias para controlar a UC por *H. ducreyi* juntamente com a bouba. Em 2012, um ensaio clínico realizado na Papua Nova Guiné mostrou que a azitromicina era eficaz para tratar a bouba ativa. No entanto, para nosso conhecimento, há dados limitados sobre a eficácia do AMM com azitromicina para curar a infeção latente da bouba ou para eliminar a UC H. ducreyi como um efeito secundário benéfico. A azitromicina tem a vantagem de ser administrada em dose oral única e a um preço muito acessível em comparação com outros regimes de antibióticos. Se comprovadamente eficaz para o tratamento de UC por *H. ducreyi* e para a bouba latente, teria implicações para o tratamento de pacientes individuais e para o uso de antibióticos em estratégias de saúde pública para controlar a UC nos trópicos. Além disso, compreender a transmissão da UC de *H. ducreyi* permitirá definir novas estratégias para controlar e deter a propagação da doença.

Os artigos originais desta tese pretendem fornecer uma atualização sobre a epidemiologia mundial da UC e bouba de *H. ducreyi*, investigar a eficácia da azitromicina de dose única no tratamento de pacientes com UC por *H. ducreyi* e bouba latente, explorar a transmissão e reservatórios ambientais de H. ducreyi e apresentar dados de eficácia a longo prazo da estratégia de AMM para eliminar a bouba e reduzir a prevalência de *H. ducreyi* a nível comunitário.

Abstract (English)

***Keywords*:** *Cutaneous Ulcer, Haemophilus ducreyi, Yaws, Treponema pallidum pertenue, Papua New Guinea.*

Cutaneous Ulcers (CU) presenting as skin lesions with loss of the superficial skin layers are commonly found in low-resource countries of Africa and the South Pacific. In certain remote and rural areas, such as the islands of Papua New Guinea, CU prevalence and morbidity is very high, hence representing an important cause of suffering and stigmatization in children. Certain CU conditions, if untreated, can result into permanent impairing deformities that will impact the person's life and anchor that person into poverty.

Although several pathogens may cause CU, two of them pose a major public health problem in the South Pacific islands, namely yaws (*Treponema pallidum pertenue*) and *Haemophilus ducreyi* and are the basis of this thesis. These two pathogens were selected for a number of reasons: first they are the two most common causes of CU in the tropical South Pacific region where the studies for this thesis were conducted. Second, these are co-endemic but its interaction as infectious diseases has not been studied. And third, because of epidemiological, clinical and therapeutic reasons the two diseases are potentially treatable in an integrated manner through total community treatment (TCT) with mass drug administration (MDA) of antibiotics.

Yaws is classified by the World Health Organization (WHO) as a neglected tropical disease (NTD) that primarily affects children 6-15 years old in communities with poor water supplies and no sanitation, and leads to chronic disfigurement and disability. In the past yaws was always considered the most common cause of CU, yet recent cohort studies conducted in Papua New Guinea (PNG), Solomon Islands and Vanuatu have identified *Haemophilus ducreyi* as an aetiologic agent causing more than half of all CU in the tropics.

In rural yaws endemic settings where there is absence of molecular diagnostics, CUs are classified on the basis of syphilis serological test results. For example, a CU with a positive serological test is classified as yaws and treated with single dose oral azithromycin, while a CU with a negative serological result is classified as non-yaws ulcers (e.g. *H. ducreyi* or other pathogens) and treated with a 5-7 day course of different wide-spectrum antibiotics.

Multiple days courses of treatment and multiple times per day dosing schedules compromise patient compliance and satisfaction.

Recently, an alarming resurgence of yaws has been observed in several countries in the tropics. The largest number of cases are reported in Papua New Guinea, Solomon Islands, and Ghana. WHO has responded to yaws resurgence by conducting Elimination Campaigns in highly endemic areas using empirical treatment of CU with single-dose oral azithromycin. If only yaws is treated during campaigns, communities might have the perception that persistence of other types of CU is related to a poor outcome of the strategy. The recent discovery that *H. ducreyi* was as a major causative agent of CU calls for further research on this matter.

New strategies to control *H. ducreyi* CU together with yaws need to be explored. In 2012, a clinical trial conducted in Papua New Guinea showed that azithromycin was efficacious to treat active yaws. However, to our knowledge there is limited data on the efficacy of azithromycin MDA to cure latent yaws infection, or to eliminate *H. ducreyi* CU as a beneficial secondary effect. Azithromycin offers the advantage of single oral dose at a very cheap price as compared to other antibiotic regimens; if proven efficacious for *H. ducreyi* CU and latent yaws, this would have implications for the treatment of individual patients and for the use of antibiotics in public health strategies to control CU in the tropics. Furthermore, understanding the transmission of *H. ducreyi* CU will helps us define new strategies to control and stop the spread of the disease.

This thesis original research articles aim to provide an update on the worldwide epidemiology of *H. ducreyi* CU and yaws, to investigate the efficacy of single-dose azithromycin to treat individual patients with *H. ducreyi* CU and latent yaws, to explore the transmission and environmental reservoirs of *H. ducreyi*, and to present long-term efficacy data of the MDA strategy to eliminate yaws and reduce the prevalence of *H. ducreyi* at a community level.

List of Abbreviations

-CFU: Colony Forming Units

-CU: Cutaneous Ulcer

-DNA: Deoxyribonucleic Acid

-GUD: Genital Ulcer Disease

-LMC: Lihir Medical Centre

-MDA: Mass Drug Administration

-NTD: Neglected Tropical Diseases

-PCR: Polymerase Chain Reaction

-PNG: Papua New Guinea

-RPR: Rapid Plasma Reagin

-TCT: Total Targeted Treatment

-TTT: Total Community Treatment

-WHO: World Health Organisation

-TPHA: Treponema pallidum haemagglutination

-NML: Newcrest Mining Limited

-ISOS: International SOS

Content Table

1 General Introduction

1.1 Cutaneous Ulcers in the Tropics

1.1.1 Introduction to Cutaneous Ulcers

The cutaneous ulcer (CU) disease is a painful and debilitating condition that anchors people into poverty in rural regions of tropical countries. It is caused by a group of bacterial infections mostly transmitted by direct skin-to-skin, nonsexual contact. CU disease typically presents as single or multiple skin lesions with epidermal loss due to sloughing out of inflamed necrotic tissue. The most common locations are the legs and arms, it is frequently chronic (i.e. >2 weeks duration), and it is often painful (1). CU is endemic in poor and remote populations in the South Pacific Islands, and in West Africa. Known predisposing factors for CUs are poor access to clean water and sanitation, and limited access to the health system. Due to a lack of consensus regarding which conditions are considered to be part of the CU disease-complex, it is hard to estimate the prevalence or incidence of this disease.

1.1.2 Management of CU and recent discoveries

Clinical features alone are insufficient to make a decision on treatment, therefore laboratory testing is often required. PCR diagnostic platforms in reference laboratories are used for confirmation of many conditions, but these facilities are remote from the communities where the diseases occur. Serology, either conducted in a peripheral laboratory or using a rapid test (e.g., Dual Path Platform (DPP) allows for serological diagnosis of yaws in the field.

In areas with limited access to healthcare, the assessment of CU is based on clinical diagnosis only. Rural settings with access to basic health care facilities can perform serological tests for confirmation of the yaws agent infection. Patients with positive syphilis serology for both treponemal and non-treponemal antibodies are considered to be yaws-CU and, those with a negative result are considered non-yaws CU. Nonetheless, serological diagnostic tools are not 100% accurate, and thus molecular methods continue to be the gold-standard on the diagnostic of CU.

Recent studies conducted in the South Pacific, suggest that *H. ducreyi* might be an important aetiologic agent of CU, reporting that this bacterium has been found in large proportion of CU in rural environments of tropical countries (1,3–7). In these studies, approximately 20%

of ulcers are caused by yaws, 50% are caused by *H. ducreyi* and the remaining 30%, are caused by polymicrobial infections.

WHO and the Papua New Guinea Ministry of Health in its National Treatment Guidelines recommend azithromycin 30 mg/kg (to a maximum of 2 g for adults) orally when yaws is serologically confirmed(2). Ulcers with a negative syphilis serology, which are caused by *Haemophilus ducreyi* (*H. ducreyi*) and other still unidentified pathogens, are typically treated with penicillin derivatives (ie, amoxicillin 500 mg 3 times daily for 5 days), chloramphenicol 500 mg 4 times daily for 7 days, or metronidazole 500 mg 3 times daily for 7 days.

The recent findings on *H. ducreyi* has implications on the management of CU and on the yaws eradication campaign led by the WHO in Africa and the South Pacific that will be discussed in this thesis. A better characterization of the CU syndrome may help to design diagnostic tools and more effective antimicrobial combination for the empirical treatment of this condition.

1.1.3 Concept and terminology

The terms used to refer to ulcerative conditions in the tropics are not common, or not well-defined in the literature and multiple communicable and non-communicable diseases can cause ulcers in tropical countries. In the light of recent findings about new causative agents (i.e. *H. ducreyi*, *C. diphteriae*) we believe that there is an opportunity to redefine the concept and terminology CUs in the tropics.

Ulcers originated from venereal infectious diseases (e.g., chancroid, syphilis, herpes) are named genital ulcers, we suggest that the term *cutaneous ulcer* or *skin ulcer*, be used to describe non-venereal skin ulcers caused by an infectious agent. Each country or region may adapt the term to the prevailing local or regional endemicity of diseases.

CUs in the tropics can be non-infectious, such as vascular or neuropathic ulcer or, more commonly, infectious. Infectious non-bacterial cutaneous ulcers include cutaneous leishmaniosis, caused by several *Leishmania* species, and some, rather rare, viral or fungal infections. The bacterial CUs group is comprised by Buruli ulcer (caused by *Mycobacterium ulcerans*), tropical ulcers (also called phagedenic ulcers, which is often referred to ulcers that are allegedly caused by Fusobacterium, Bacillus fusiformis, Treponema vincentii, Escherichia coli and Enterococcus), yaws (caused by *Treponema pallidum pertenue [T. p.*

pertenue]), cutaneous diphteria (caused by *Corynebacterium diphteriae*), *Haemophilus ducreyi*, and a sixth group of idiopathic ulcers of still unknown cause.

For the purpose of this study, we will use the term CU to refer to bacterial ulcers which are endemic in our study setting: 1) yaws, 2) tropical ulcers, 3) cutaneous diphteria 4) *H. ducreyi* ulcers and 5) idiopathic ulcers.

1.2 Haemophilus ducreyi: Introduction

Panel 1. Key Facts

- ***H. ducreyi* is a gram-negative bacterium widely known as causative agent of genital ulcerative disease (GUD) chancroid.**
- **After the implementation of Syndromic Management for GUD in the 1950's the prevalence of chancroid dramatically decreased worldwide.**
- ***H. ducreyi* has been recently identified as a main aetiologic agent of CU in the South Pacific and West Africa.**
- ***H. ducreyi*-CU and yaws are co-endemic, and both pathogens affect mostly the limbs of children aged 5 to 15 years old.**
- **Clinical assessment of *H. ducreyi*-CU is unreliable for diagnosis; laboratory tests continue to be recommended.**
- ***In vitro* and *in vivo* studies show *H. ducreyi* to be susceptible to macrolides, including azithromycin.**

H. ducreyi is commonly known as the causative agent of genital ulcer disease (GUD), chancroid. There is an enormous knowledge gap regarding current epidemiological information of the disease. In the 1950s, the WHO launched a campaign to implement syndromic management for bacterial GUD (i.e., treatment with antimicrobial drugs effective against syphilis and chancroid). Currently GUD is a notifiable disease and number of cases per annum are reported to WHO on the basis of a clinical diagnosis without laboratory testing which makes it hard to evaluate the true incidence of *H. ducreyi* -GUD worldwide.

1.2.1 Epidemiology and Geographic distribution

H. ducreyi CU has been found in rural areas in communities living with poor hygiene. Children wearing scanty clothing, sharing bed and linens (which is a common practice in PNG), lack of soap and current water for bathing are all thought to favour transmission.

As in yaws, the incidence of *H. ducreyi* CU is higher in the age group 5 – 19 year-old children, while children below the age of 4 and older adults are less affected (3). Transmission can happen when children are playing or sharing school desks or bed. *H.*

ducreyi skin ulcers have a male predominance which is related both to biological factors of the host and to the more physical forms of exercise done by males that predispose them to minor trauma. *H. ducreyi* CU is transmitted through non-intact skin contact with infectious lesions.

The geographic distribution of *H. ducreyi*-associated CU is still unclear. Earlier studies of tropical skin ulcers did not generally test for *H. ducreyi*, with the exception of a small number of case reports. There are major limitations in describing the prevalence or incidence of causative agents in tropical CU that typically occur in children in rural areas where there is no access to laboratory facilities. Even where laboratory facilities are available, microbiology techniques usually comprise Gram staining of exudative material; for example, *Fusobacterium fusiforme*, and *Staphylococcus aureus* have been seen in some cases. However, culture or PCR testing for definitive identification of fastidious pathogens, such as *H. ducreyi*, has not been conducted.

1.2.1 Emergence of *H. ducreyi*-caused Cutaneous Ulcers in the South Pacific

The first case of non-sexual *H. ducreyi* infection was reported in 1988, on a traveller returning from Fiji islands(8). This patient presented with a chronic foot ulcer secondary to a swimming injury. Subsequently seven other cases, 4 children and 3 adults, where reported; all of them presenting with leg ulcers after travels to the South Pacific Islands and Territories (8–12). The real extent of *H. ducreyi* CU was demonstrated in a number of community surveys conducted in yaws-endemic areas of PNG Vanuatu, Solomon Islands, and Ghana. Mitjà et al. conducted a prospective cohort study in PNG in 2013, and found that 54 (60%) of 90 participants with skin ulcers were positive for *H. ducreyi* on PCR (3), whereas 31 (34%) cases had *T. p. pertenue* DNA; and 12 (13%) cases had mixed *H. ducreyi* and *T. p. pertenue* infection. School-children were most commonly affected. Further studies undertook in Solomon islands, Vanuatu and Ghana, reported *H. ducreyi* in up to 40% of all the CU molecularly tested.

1.2.2 Pathogenesis and risk factors

H. ducreyi is a gram-negative bacterium (13) and obligate human pathogen that enters the human host through small breaks in the skin (15). GU strains are mainly transmitted by direct

sexual contact following disruption of the genital epithelial surface (14). The clinical and microbiological similarities of *H. ducreyi* CU strains suggest that pathogenesis might be the same in CU as it is in GUD.

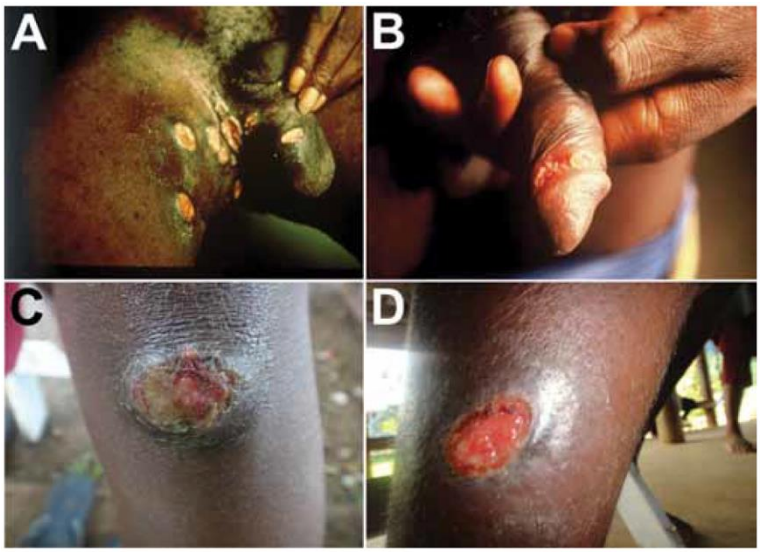

Figure 1: Ulcers caused by infection with *Haemophilus ducreyi*. A, B) Genital ulcers in adult patients from Ghana (provided by David Mabey). C, D) Skin ulcers in children from Papua New Guinea (provided by Oriol Mitjà). Ref: González-Beiras et al. Single dose azithromycin for the treatment of Haemophilus ducreyi skin ulcers in Papua New Guinea. Clin Infect Dis . 2017;

In human experimental models as few as 1 colony forming units (CFU) of the genital ulcer strain 35000HP is able to infect the skin after being delivered by a puncture wound; however, placement of up to 10^6 CFU of *H. ducreyi* on intact skin fails to cause infection (16), which suggests a small wound is needed to initiate infection. After initial infection with *H. ducreyi*, the infection develops into a pustule in 70% of volunteers while it resolves spontaneously in the remaining 30%. Several factors are suspected to impact the initiation of disease after infection, such as the composition of the skin microbiome (17), or gender (in experimental studies men are twice as likely as women to develop disease) (18). Importantly, infection with *H. ducreyi* does not confer protective immunity against subsequent exposure to the organism (19).

1.2.3 Evolutionary genetics of *H. ducreyi* CU strains

Ganghaiah et al, performed whole-genome sequencing to determine the relatedness of the *H. ducreyi* strains causing CU to those *H. ducreyi* strains causing GUD (20), the study showed that cutaneous strains obtained from Samoa and Vanuatu are genetically nearly identical to class I type genital strains. Class I and Class II strains can be found in both genital

and cutaneous strains. A recent analysis reports that cutaneous *H. ducreyi* strains diverged from class I genital strains approximately 0.18 million years ago (21).

1.2.4 *H. ducreyi* and yaws geographical overlap

H. ducreyi skin infections often occur in yaws endemic areas (5-9). Both diseases present very similar phenotypical appearance and demographic characteristics (3). Lesions caused by *H. ducreyi* are extremely difficult to distinguish clinically from yaws lesions and may be found in patients who are both sero-positive (i.e., children with a dual infection) and sero-negative for yaws. *H. ducreyi* DNA has been found alongside *T. p. pertenue* DNA in yaws like lesions in several studies. Three to 13% of children with *H. ducreyi* positive ulcers were either co-infected with *T. p. pertenue* or had serological evidence of past infection with yaws. It is thought that *T. p. pertenue* may cause the initial break in the skin that permits entry of *H. ducreyi.*

1.2.5 Clinical features

H. ducreyi skin infection presents as a single ulcer or multiple ulcers localized in a region of the body, *H. ducreyi* ulcer can present as deep or shallow, commonly irregular in shape, and does not usually involve subcutaneous tissue (3).

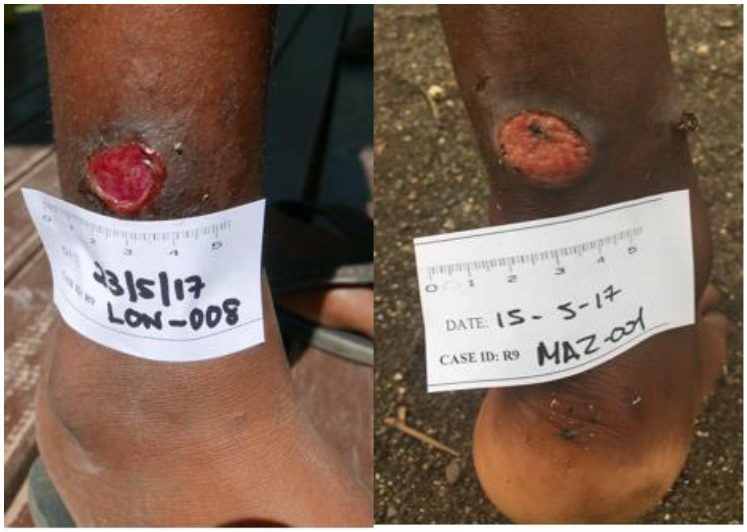

Figure 2: Cutaneous ulcers caused by *Haemophilus ducreyi.*

The size of the ulcers varies in range from 0.5cm to 4 cm (median 2.1cm). Clinically the ulcers are noted to spread without elevation or undermining of the edges. The base of the ulcer is most frequently clean granulation tissue and dry, sometimes there is necrotic tissue

debris, and occasionally serous or purulent exudate. Pain has been associated but not uniformly reported. Untreated lesions usually heal spontaneously after several weeks. No complications such as osteomyelitis, deep tissue abscess or gangrene have been noted as a consequence of an ulcer.

H. ducreyi ulcers predominantly appear on the lower limbs; they are infrequently observed above the knee or the arms. On the leg, the locations usually involve parts most susceptible to trauma, such as bony prominences, like the ankles. The number of ulcers per patients varies from 1 to multiple ulcers. Spontaneous satellite lesions or ulcers related to additional scratching or trauma can be seen.

1.2.6 Diagnosis

Clinical diagnosis of cutaneous *H. ducreyi* ulcers can be challenging due to their similarity to other bacterial ulcerative diseases which highlights the need for laboratory diagnostic testing (3). *H. ducreyi* may be mistaken for several other diseases such as primary yaws, buruli ulcer, cutaneous leishmaniasis, pyoderma or tropical ulcer caused by anaerobic bacillus. *H. ducreyi* ulcers are smaller, less circular, and less likely to have central granulating tissue or indurated edges than yaws ulcers and ulcers caused by other aetiological agents. However, this clinical distinction is unreliable except in classical cases.

Traditionally, culture on selective agar plates has been the gold standard for *H. ducreyi* diagnosis, yet this method is technically difficult and insensitive. Molecular methods are now preferred for identification of *H. ducreyi*, though are rarely available in developing countries where *H. ducreyi* skin ulcers occur (22).

H. ducreyi is a fastidious organism requiring specific nutritional, atmospheric, and temperature requirements to be cultured (31). The use of freshly-made media with vancomycin and attention to the incubation temperature 33 – 35ºC is critical for successful cultivation. The colonies present with a characteristic morphology, and can be pushed intact across agar surfaces. Identification relies on biochemical reactions, and on microscopy the bacteria manifests as ‘schools of fish’ because of the organism clumping. Culture-based techniques are still required for determination of antimicrobial susceptibility patterns. Minimum inhibitory concentrations of antimicrobial agents may be determined by conventional agar dilution.

Several PCR assays have been developed for detection of *H. ducreyi* in clinical samples that amplify DNA sequence from a number of targets on the *H. ducreyi* genome, including the 16S rRNA gene, the groEL gene, and the hemolysin gene. Multiplex PCR to simultaneously detect the 3 major causes of genital ulcer disease (*H. ducreyi, T. pallidum* and HSV types 1 and 2) is frequently used. However, a multiplex PCR to detect the major causes of skin ulcers has not yet been developed.

1.2.7 Treatment

All *H. ducreyi* strains causing skin ulcers tested so far have been susceptible to penicillin, amoxicillin, ceftriaxone, doxycycline, ciprofloxacin, azithromycin, and cotrimoxazol (8,9,20). The CU strains of 4 travellers from Samoa, 1 from Vanuatu and 1 from Fiji have been shown to have similar susceptibility patterns as the genital type strain 35000HP. Almost all the genital strains are resistant to penicillin (>256 fold), while the 35000HP and all CU strains have been b-lactamase negative with penicillin minimum inhibitory concentrations of 0.25 mg/l.

Clinical data about the use of antibiotics to treat *H. ducreyi* CU is very limited. Also, the absolute number of ulcer cases found to be *H. ducreyi* DNA positive following mass azithromycin treatment interventions in several locations was found to be significantly lower compared to pre-intervention levels (1). However, definitive clinical data on the efficacy of antibiotics to treat *H. ducreyi* CU is still lacking.

Non-yaws CU in most resource-poor countries are normally treated empirically with penicillin derivatives. Based on drug susceptibility testing data, cutaneous *H. ducreyi* strain would have responded well to B-lactams. Typically, injectable benzathine penicillin IM 1.2 MU (0.6 MU for children under 12 years) is used when yaws is suspected.

Azithromycin offers the advantage of single dose oral therapy, and it was recently demonstrated to be as effective as benzhatine penicillin for treatment of yaws ulcers. Azithromycin has good in vitro activity against *H. ducreyi* genital ulcer strains (24,25), it has been clinically demonstrated to be an effective treatment for chancroid (23), and CU strains from patients infected in Samoa, Vanuatu and Fiji have been susceptible to this antibiotic (20).

1.2.8 Prevention and control

Several studies have recently confirmed that high coverage community mass azithromycin treatment, given as a single oral dose of 30mg per kg, up to a maximum dose of 2 g has less impact on lesions caused by *H. ducreyi* than those caused by *T. p. pertenue.* Although all *H. ducreyi* strains causing CUs tested have been susceptible to azithromycin (20), and a single 1 g dose of azithromycin prevents disease from experimental inoculation for nearly 2 months (26), the findings of *H. ducreyi* persistence in communities after azithromycin mass treatment are puzzling. There are several possible explanations. Firstly, *H. ducreyi* strains that cause skin lesions in children are probably more infectious than *T. p. pertenue*, and chancroid appears to be more easily transmitted than syphilis. In addition, natural infection with *H. ducreyi* does not appear to induce any immunity to subsequent infection, unlike the transient immunity that occurs following treponemal infection (8). Furthermore, we hypothesise that colonization of non-genital skin in asymptomatic villagers and the possible carriage of bacteria by the flies may contribute to the continued presence of infection after preventative interventions. Skin-colonizing bacteria that escape systemic treatment could infect the skin after a minor abrasion and may serve as a reservoir for transmission to others.

New strategies to control *H. ducreyi* need to be explored. Understanding the transmission of this pathogen will helps us define new strategies to control and contain the spread of the disease. Past studies on yaws have emphasized the salutary effect of soap and water on yaws prevalence. It is quite possible that improved hygiene, coupled with modified-MDA to confer longer prophylactic effect to the treated population, could reduce *H. ducreyi* infection.

1.3 Yaws

Panel 2: Key facts

- **Yaws is a neglected disease of the skin and bones caused by *Treponema pallidum pertenue.***
- **Mass campaigns in the 1950-1960's in 46 countries, reduced the worldwide prevalence by 95%.**
- **Yaws can still be found in remote rural regions of some low-resource countries.**
- **Yaws mainly affects children 5-15 years of age.**
- **Single oral dose of azithromycin is effective to treat yaws.**
- **Yaws can be eliminated and eventually eradicated because humans are the only reservoir of infection.**

Yaws, is a non-venereal infectious disease caused by *T p. pertenue* that occurs primarily in children less than15 years of age in rural, humid, tropical regions, causing painful ulcers in the skin. If untreated it can lead to destructive lesions of the bone. This disease is highly infectious and easily transmitted through skin-to-skin contact.

1.3.1 Pathogenesis

T p. pertenue is morphologically and serologically indistinguishable from the bacterium *Treponema pallidum pallidum,* causative agent of venereal syphilis. Although, unlike syphilis, yaws is transmitted thought direct contact with exudative skin lesions of infected people (27). The organism presumably enters the human host through small breaks in the skin; it appears in the lymph nodes within minutes and disseminates widely within hours (28). Treponemes move through epithelial cells via tight junctions and invasively attach to fibronectin-coated surfaces on the extracellular matrix of host cells (2).

The yaws treponemes are found mostly in extracellular clusters in the upper regions of the epidermis, unlike treponemes in the subspecies pallidum, which are located mainly in the

dermis and dermal–epidermal junction The host responds to yaws infection with both humoral and cellular immune responses (29).

1.3.2 Clinical features

Primary lesions usually appear in legs and ankles as a localised papule which can develop into a large papilloma 2–5 cm in diameter (29). Secondary lesions appear a few weeks to 2 years after the primary infection and present as multiple skin lesions with or without bone pain. Bone lesions are common and occur in numerous bones simultaneously in early stages. Spontaneous healing occurs within a few months without treatment. In rare cases, after more than 5 years of untreated infection, stage 3 will manifest with destruction of the bone and deformity.

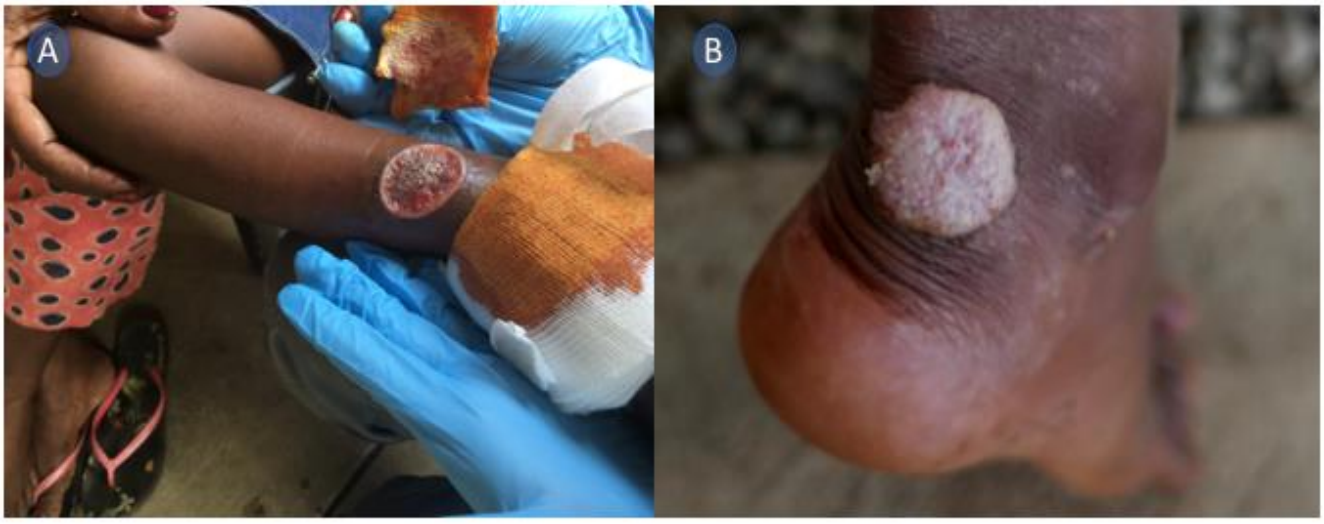

Figure 3: A) Cutaneous ulcer caused by *T.p.pertenue* and B) Papilloma caused by *T.p.pertenue*

1.3.3 Epidemiology

Yaws is found in warm and humid environments (30) 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 (27). Children born to mothers affected with yaws are generally unaffected, and most evidence seems to indicate that the disease is not acquired congenitally (31). The early lesions of yaws are most infectious. It is estimated that patients are infectious for up to 12–18 months

following primary infection (27), but relapsing disease can extend this period. The destructive lesions of late yaws are not infectious. In studies in both PNG and the Solomon Islands, endemicity at the village level has been identified as the major risk factor for infection and re-infection following treatment.

During 2010–13, over 250.000 yaws cases were reported to WHO from 13 endemic countries, all of which are low-income and middle-income countries. 84% of cases reported to WHO were from three countries—PNG, Solomon Islands, and Ghana.

The global prevalence of yaws is likely underestimated, but ~ 84 million people live in 13 yaws endemic countries. In rural endemic regions of Africa and the South Pacific islands, ~ 5 to 15% of children have exudative CU that are attributed to *T. p. pertenue*. Yaws is classified by the World Health Organization (WHO) as a Neglected Tropical Disease (NTD) and primarily affects children 6-15 years old in communities with poor water supplies and no sanitation, and leads to chronic disfigurement and disability (32). From its inception, WHO has led several campaigns using injectable penicillin to eliminate yaws, but the global prevalence of CU remains substantial, with ~100 million children at risk for infection (32,33).

Because *T. p. pertenue* is temperature and humidity dependent, yaws is found in warm, moist climates, mainly in forested tropical regions (29). Yaws predominantly affects children younger than 15 years, and children aged 2–15 years are the main reservoir of infection. Although the disease can be clustered in households, transmission often between children in the community, schools, and other public places

Yaws is believed to be a strictly human pathogen, although the pertenue subspecies has been found affecting non-human primates, such as gorillas in the Democratic Republic of the Congo, and studies show that experimental inoculation of human beings with a simian isolate causes yaws-like disease. However, there is no evidence of cross-transmission between humans and primates (29).

1.3.4 Diagnosis

Primary yaws is frequently confused with a number of conditions common in the tropics (29), and thus it is possible that a significant proportion of cases may in fact be falsely diagnosed as yaws on clinical grounds. Health-care workers who are not familiar with the

diseases might under-report or over-report yaws, unless serological or molecular diagnosis is available.

Dark-field microscopy or by immunohistochemistry from biopsy specimens can identify the treponemes, although these rarely used nowadays to diagnose treponemal infection.

Serologic tests include the non-treponemal tests (such as rapid plasma reaging RPR test) which determine the disease current vs past/treated status, and the treponemal test (such as T. pallidum hemagglutination assay TPHA). Non-treponemal tests are positive in untreated cases and can be used as test of cure because they usually revert to negative after successful treatment while treponemal tests are more specific but remain positive for life, even after successful treatment. A new rapid point-of-care rapid diagnostic test (RDT) which can run treponemal and non treponemal analysis has been recently developed (34).

The inability to serologically differentiate yaws and syphilis can be an issue in countries where both diseases are endemic, since the existing serological test cannot differentiate among the two treponemes subspecies. Real time PCR is useful test to differentiate the two treponemal diseases, however, this technique is expensive and rarely available in rural endemic communities (29). In these settings, traditional RPR tests will continue to be used while taking into consideration the epidemiological and demographic characteristics of yaws, as for the clinical history and demographic characteristics of the patient.

1.3.5 Treatment

Injectable penicillin was the recommended treatment for yaws disease for decades. Long-acting Benzathine penicillin G, given intramuscularly was, and still is, one of the recommended treatments. The recent discovery that one single oral dose of azithromycin is as effective as the intramuscular penicillin has promoted modification of first line treatment to azithromycin because of ease of administration (35).

Yaws CU become non-infectious 24 hours after treatment and RPR titres decline within 6-12 months. A small proportion of cases may remain positive, although with low titres (>1:8) in what is known as serofast stage.

Unlike *H. ducreyi* CU, infection with yaws disease usually confers resistance for future infections, although reinfections are not uncommon. The distinction between reinfection, relapse or true resistance is difficult to formulate.

1.4 Study area and population.

The study area is located in Lihir Island, New Ireland Province, Northern Islands of PNG (Figure 1).

PNG is the largest country of the Pacific region. The indicators of health status show PNG as a seriously disadvantaged country in the region across a number of health measures: one-third of its 7 million population live below the national poverty line, 87% of the population lives in rural areas, and only 33% of this rural population have access to a safe water source. Much of the country is only accessible by air and less than 5 percent of roads are usable year-round (36).

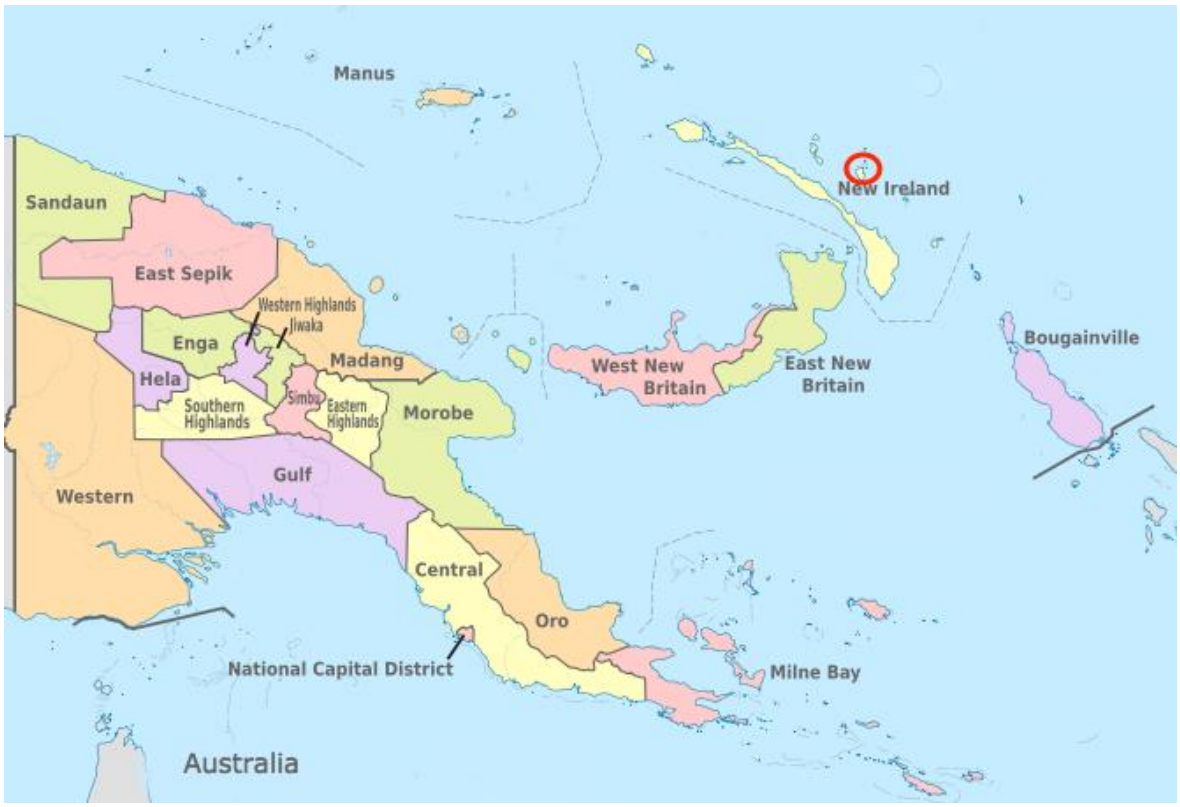

Figure 1: Map of Papua New Guinea. (Lihir Island in red circle)

Lihir is a volcanic mountainous island rising 700 metres above sea level with most people living close to the coast. Lihir is 22 kilometres long and 15 kilometres wide, and experiences high annual rainfall with occasional flooding from heavy rains. Seasonal dry periods occur and water shortages are not uncommon. The climate is warm to hot (average temperature of 28 degrees Celsius) with a relative humidity of 80%. Lihir has a partially sealed and maintained ring road that runs around the island. Public transport is available either by the bus services or in the back of larger community owned trucks. Many people walk along the

roads while carrying out their daily affairs. Local rivers and creeks are used for personal washing, preparing food and washing utensils.

The smaller outer islands have little or no civil administrative or social service infrastructure. There are no public vehicles; people usually get around on foot or by canoe. Motorised banana boats are the main transport to and from neighbouring islands. There are at least twice daily services to the main island, except when the seas are rough and unsafe to travel on (not uncommon). Most people from the outer islands travel to and from the main island for employment, shopping, trading food produce, visiting family and friends and to access essential services, including health care. The majority of the population lives in small villages without access to clean water or sanitation measures (Image 1) (37)

Figure 4:: Village of Lihir Island (Kunayie).

The population living in Lihir Island is of around 25.608 of which almost 14.000 are non Lihirians. 40% of the population are children under 15 years of age. (38)

Lihir island has one public health centre (Palie Health Centre) situated in the South-West coast of the island, which offers free but extremely limited health care services to the inhabitants of the island, and several aid-posts allocated around the island which provide primary care and manage several uncomplicated infectious diseases such as malaria or yaws (Figure 5).

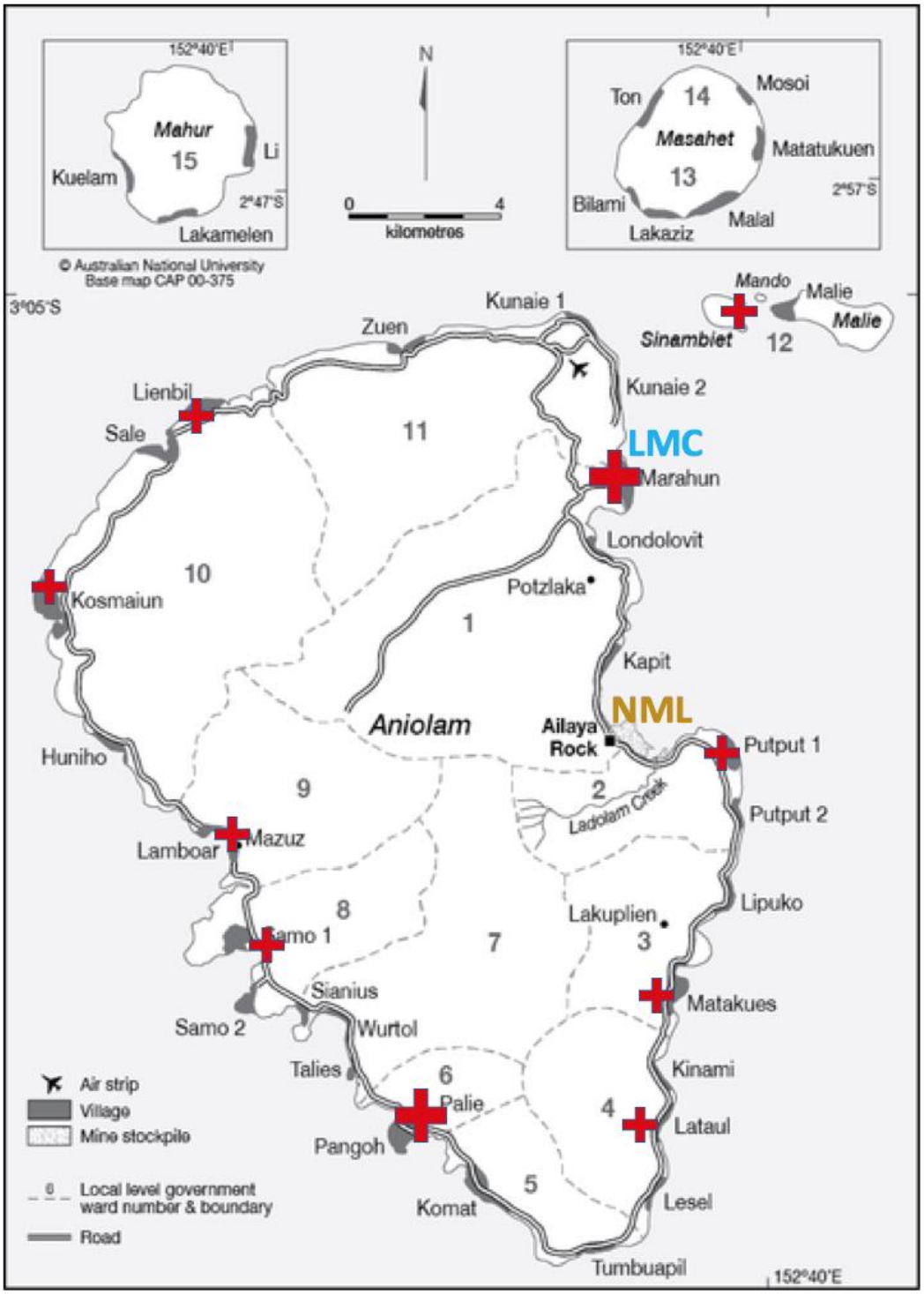

Figure 5: Map of Lihir island. (NML: Newcrest Mining Limited; LMC: Lihir Medical Centre; red crosses mark location of aid-posts and health centers).

Furthermore, Lihir island has 22 schools (primary and elementary), scattered along the coast of the whole island. Collaboration with school teachers and health centre nurses was key to the successful development of our research projects. (Figure 6)

Figure 6 Lihirian children at school door.

This research was undertaken at the Lihir Medical Centre (LMC), a private health centre located in Londolovit village, in the North-Eastern part of the Island (Figure 3). LMC was set up with the mission of providing curative service to the workers of Newcrest Mining Limited (NML) gold mine, which has been active on the island since 1997 (Figure 3), and the Lihir Island population. LMC is sub-contracted and managed by International SOS (ISOS) and funded by NML.

LMC has modem health facilities and provides services such as outpatients, inpatients, dispensary, medical laboratory, dental services, radio imaging, operating theatre and labour ward. LMC has a program of community health that conduct outreach programs to deploy preventive interventions such as MDA programs, immunization programs, vector control and bed-net distribution programs, etc. The community health department also supports research projects under a Memorandum of Understanding with the Barcelona Institute for Global Health. The LMC community health team was actively involved in the research projects related to this thesis

1.5 Specific introduction to this Thesis

This thesis presents the work done in Lihir Island, PNG, during a collaborative project between the Lisbon Institute of Hygiene and Tropical Medicine (IHMT) from the Nova University of Lisbon, Portugal and the Barcelona Institute for Global Health (ISGlobal), Spain, as dissertation thesis for the Global Public Health PhD programme by the IHMT, Nova University of Lisbon.

The research studies on this thesis are the result of extensive research on CUs affecting remote endemic populations of PNG. We focused our research on the two major co-endemic CU diseases: yaws, caused by *T. p. pertenue*, and *Haemophilus ducreyi*.

Studies I and **II** are systematic reviews on the global epidemiology of yaws and *H. ducreyi* infections worldwide. These papers serve as an approximation to the state of art of CU epidemiology and an update onto the current knowledge on these two disease.

Studies III, IV, V and VI are all nested studies within a large longitudinal study (**Study VII**) including more than 16,000 residents to assess the impact of a population-wide mass azithromycin treatment intervention to eliminate yaws in Lihir Island. The longitudinal study consisted of a before- and after- intervention assessment by means of repeated cross-sectional surveys to identify CU cases in the communities. Different subsets of participants presenting with CU and meeting specific criteria were enrolled in sub-cohort studies for follow up overtime in studies III and IV. We also used the specimens collected that met specific criteria to do further testing for study V, finally we identified asymptomatic participants related to CU-participants to assess colonization in study VI.

Studies III and IV assessed the efficacy of a single oral dose of azithromycin to treat *H. ducreyi*-CU and latent yaws, respectively. **Study III** results from a rigorously conducted prospective cohort study that followed up individual patients to assess clinical cure, while **Study IV** results from a prospective cohort study to assess for serological cure.

Studies V and **VI**, focus on the transmission and reservoirs of *H. ducreyi* strains in our research setting. **Study V** reports the results of a molecular analysis on the *H. ducreyi* CU strains obtained at different points before and after an azithromycin MDA intervention, and of selective pressure analyses carried out to evaluate the impact of MDA on *H. ducreyi* cutaneous strains. **Study VI** is a cross-sectional study performed to evaluate the colonization

capacity of *H. ducreyi,* as the ubiquity of *H. ducreyi* in the environment appears to be a factor on the resilience and transmission of the organism.

Study VII presents data on the prevalence of yaws disease in Lihir island over a 42 months period after implementation of the Morges strategy (azithromycin MDA followed by biannual rounds of active case search) in a longitudinal study including more than 16,000 residents.

1.5.1 Study I

Between 1952 and 1964, WHO and the United Nations Children's Fund (UNICEF) led a multinational worldwide campaign to control and eventually eradicate yaws through mass treatment by means of community-wide treatment with long acting, injectable penicillin which reduced the number of cases of the disease by 95% worldwide. However, this approach did not succeed. Our first study, reports the resurgence of yaws during the past decade in Western and Central Africa, Southeast Asia, and the Pacific Islands.

1.5.2 Study II

So far the epidemiology of *H. ducreyi* infections has been poorly documented, mainly due to the difficulties in confirming microbiological diagnoses in the settings were the disease occurs. Study II was performed through the revision of published data on the proportion of genital ulcers caused by *H. ducreyi* among all-cause ulcers before and after the introduction of syndromic management for GUD. The study provides a clear perspective of the dramatic reduction on the proportion of GUD caused by *H. ducreyi* and, most importantly, of the emergence of *H. ducreyi* as a major cause of non-genital CU in the tropics.

1.5.3 Study III

The third paper is a population based cohort study that evaluates the effectiveness of azithromycin to treat *H. ducreyi* CU. Recent studies evaluating the effectiveness of azithromycin to treat yaws in the context of MDA, reported a persistence of *H. ducreyi* ulcers after MDA raising concerns about the efficacy of this antibiotic to treat *H. ducreyi*. This paper presents for the first time, evidence that azithromycin is highly effective to treat *H. ducreyi* CU, which has implications for the treatment of individual patients and for the use of antibiotics in public health strategies to control CU in the tropics. Persistence of *H. ducreyi* ulcers after MDA, is most likely related to other reasons.

1.5.4 Study IV

The fourth paper on this thesis reports the results of a cohort study designed to assess the effectiveness of single dose azithromycin to treat latent yaws, on the basis of serological response after 12-24 months. Study IV presents strong evidence on the efficacy of azithromycin to treat latent yaws infection which has implications at individual level, because the antibiotic can prevent the progression of the infection to the destructive tertiary stage, and at a community level, because it can prevent relapsing episodes resulting in the transmission to uninfected children.

1.5.5 Study V

The fifth paper is a molecular study of the *H. ducreyi* CU strains from swabs collected over a long period of time. First, we developed a cost-efficient system to type samples by examining which components of the multi locus system are required to discriminate between CU strains and types. Second, we aimed to evaluate the selective pressure exerted by the MDA of the yaws elimination programme on *H. ducreyi* CU. The paper evaluates the dynamics of transmission of *H. ducreyi* overtime, and shows no changes on *H. ducreyi* strains distribution and diversity

1.5.6 Study VI

The sixth study is the result of a cross-sectional survey to investigate *H. ducreyi* environmental reservoirs. *H. ducreyi* DNA was found to colonize the skin of healthy subjects and on flies and bedsheets. The ubiquity of *H. ducreyi* is a contributing factor on the spread of the organism, which can explain its persistence after MDA interventions.

1.5.7 Study VII

The last study reports the long-term efficacy of the modern WHO strategy for yaws eradication (Morges strategy). Through an entire-population screening survey approach we were able to accurately measure the prevalence of active yaws, measure the effect of treatment on latent infection, determine the molecular diversity of *T. p. pertenue* infections, and detect the appearance of macrolide resistant strains

2 Hypothesis and objectives

2.1 Hypothesis

Study I

- The MDA campaigns to eliminate yaws implemented by WHO and UNICEF in the 1950's substantially reduced the prevalence of the disease worldwide.
- Yaws has re-emerged in the last 20 years in some of the previously endemic countries of West Africa and the South Pacific.

Study II

- *H. ducreyi* used to cause a large proportion of genital ulcer disease before the global implementation of syndromic treatment for genital ulcers.

Study III

- *S*ingle dose oral azithromycin is effective to treat *H. ducreyi* CU measured by clinical healing of the skin lesions.
- *H. ducreyi*- PCR results revert to negative after treatment with azithromycin.

Study IV

- Asymptomatic patients with latent yaws (i.e. positive serology) present serological cure defined as a four-fold decline in RPR titre or seroreversion at 12-24 months.

Study V

- Class I and class II CU strains circulate on Lihir island and dual infections (Class I and Class II) occur in this population.
- The yaws elimination program exerted selective pressure on and changed the composition or distribution of circulating *H. ducreyi* strains over time.

Study VI

- *H. ducreyi* DNA can be found on the skin of healthy subjects, flies and fomites**.**
- *H. ducreyi* can remain present in the environment even after MDA with azithromycin.

Study VII

- One single round of total community MDA with azithromycin is enough to interrupt

transmission of yaws disease in an endemic community.

2.2 General objectives

1. To study the global epidemiology of CU diseases worldwide.
2. To assess the effectiveness of single dose oral azithromycin to treat CU diseases.
3. To understand the mode of transmission of CU diseases in order to improve supportive preventative measures.

2.3 Specific objectives

1 To update the worldwide epidemiology of yaws disease.

2 To improve our understanding of the worldwide epidemiology of *H. ducreyi* infections

3 To assess the clinical response of CUs caused by *H. ducreyi* after treatment with azithromycin.

4 To examine the serologic response of subjects with latent yaws infection after treatment with azithromycin.

5 To map the genetic population structure of *H. ducreyi* and to analyse the transmission patterns of *H. ducreyi* using molecular methods.

6 To develop a cost-efficient system to type *H. ducreyi* samples from resource poor areas.

7 To analyse the colonization capacity of *H. ducreyi* after antibiotic MDA.

8 To assess whether a single round of azithromycin MDA followed by bi-annual active case search is sufficient to eliminate yaws from an endemic community.

3 Results

3.1 Study I

Global epidemiology of yaws: a systematic review

Oriol Mitjà, Michael Marks, Diby J P Konan, Gilbert Ayelo, Camila González -Beiras, Bernard Boua, Wendy Houinei, Yiragnima Kobara, Earnest N Tabah, Agana Nsiire, Damas Obvala, Fasiah Taleo, Rita Djupuri, Zhang Zaixing, Jürg Utzinger, Lasse S Vestergaard, Quique Bassat and Kingsley Asiedu.

Lancet Global Health. Jun 2015. 3(6), pp.e324-e331. [IF 17.68]

3.2 Study II.

Epidemiology of Haemophilus ducreyi Infections.

Camila González-Beiras, Michael Marks, Cheng Y Chen, Sally Roberts, and Oriol Mitjà.

Emerging Infectious Diseases, January 2016. 22(1), pp.1-8. [IF 6.7]

3.3 Study III

Single dose azithromycin for the treatment of Haemophilus ducreyi skin ulcers in Papua New Guinea.

Camila González-Beiras, August Kapa, Marti Vall Mayans, Raymond Paru, Sergi Gavilán, Wendy Houinei, Sibauk Bieb, Sergi Sanz, Rosario Martíns, Oriol Mitjà

Clinical Infectious Diseases, November 2017. v 29;65(12):2085-2090 [IF 8.9]

3.4 Study IV

Effectiveness of single-dose azithromycin to treat latent yaws: a longitudinal comparative cohort study

Oriol Mitjà, Camila González-Beiras, Charmie Godornes, Reman Kolmau, Wendy Houinei, Haina Abel, August Kapa, Raymond Paru, Sibauk V Bieb, James Wangi, Sergi Sanz, Kingsley Asiedu, Sheila A Lukehart, Quique Bassat

Lancet Global Health. December 2017. 5(12):e1268-e1274 [IF 17.8]

3.5 Study V

Multiple class I and class II Haemophilus ducreyi strains cause cutaneous ulcers in children on an endemic island.

Grant, JC*, Camila González-Beiras, * **,** Fortney, KR., Gangaiah, D., Humphreys, TL., Amick, KM., Mitjà, O., Abecasis, A., and. Spinola, SM***Co-first authors**

Clinical Infectious Diseases.2018 (In press). [IF 8.9]

3.6 Study VI

Haemophilus ducreyi DNA is detectable on the skin of asymptomatic children, flies and fomites in villages of Papua New Guinea

Wendy Houinei Charmie Godornes, August Kapa, Sascha Knauf, Eric Q. Mooring, Camila González-Beiras, Ronald Watup, Raymond Paru, Paul Advent, Sivauk Bieb, Sergi Sanz, Quique Bassat, Stanley M. Spinola, Sheila A. Lukehart and Oriol Mitjà.

PLOS Neglected Tropical Diseases. May 2017. 11(5), p.e0004958. [IF 3.95]

3.7 Study VII

Re-emergence of yaws after single mass azithromycin treatment followed by targeted treatment: a longitudinal study

Oriol Mitjà, Charmie Godornes, Wendy Houinei, August Kapa, Raymond Paru, Haina Abel, Eric Q. Mooring, Camila González-Beiras, Sivauk V. Bieb, James Wangi, Sergi Sanz, Alyssa E. Barry, Kingsley Asiedu, Quique Bassat, Sheila A. Lukehart.

The Lancet, February 2018 (Available Online) [IF 44]

4 Conclusions and Discussion

4.1 Conclusions, main results and limitations:

4.1.1 Study I

Global epidemiology of yaws: a systematic review

Main results

- The MDA campaigns to eliminate yaws implemented by WHO and UNICEF in the 1950's substantially reduced the prevalence of the disease worldwide.
- Yaws is endemic in 13 countries, and at least19 other countries have an unknown endemicity status.
- At the time of this study, there were over 65.000 annual reported cases of yaws.
- 85% of all infections occurred in three countries: Ghana, PNG, and Solomon Islands
- We estimated that a maximum of about 89 million people were living in yaws-endemic areas among the 13 known endemic countries

Conclusions

- There has been limited progress towards the elimination of the disease since the 1950's campaigns.
- Due to lack of surveillance, yaws has resurged in several countries.
- PNG, Solomon Islands, and Ghana should be the focus of initial efforts at implementing the WHO yaws eradication strategy.
- Community-based mapping and active surveillance must accompany the implementation of yaws eradication activities.

Limitations

- Yaws is not a notifiable disease in many countries and the use of national routine surveillance data in our study is likely to result in an under estimation of the real number of cases.
- The limited accuracy of clinical diagnosis of yaws and the recognition that other organisms can cause clinically similar skin lesions in yaws-endemic countries means that clinical case reporting is unrealiable.

4.1.2 Study II

Epidemiology of Haemophilus ducreyi infections

Main results

- Chancroid was a highly prevalent GUD worldwide before the implementation of syndromic management.
- Implementation of syndromic management for GUD resulted in a dramatic reduction in the proportion of GUD related to *H. ducreyi* worldwide.
- There is increasing evidence of *H. ducreyi* being a newly recognised causative agent of CU in children.

Conclusions

- Over the past decade the levels of proportion of GUD caused by *H. ducreyi* have remained low, but a the bacteria has been found to be the causative agent of CU disease in the tropics.

Limitations

- The increase in HSV-related GUD as a result of immunosuppression by HIV infection could have resulted in a decrease in the proportion of chancroid among all GUD case-patients.
- The lack of sequential studies performed in similar clinical settings at multiple time points precludes an optimal interpretation of the apparent decrease.
- Results might be affected by poor-quality data from many developing countries and might be inflated by publication bias.
- PCR is more sensitive than culture. Therefore, increasing diagnostic yield might have partially masked the scale of the decrease in *H. ducreyi* as a cause of GUD.

4.1.3 Study III

Single-Dose Azithromycin for the Treatment of Haemophilus ducreyi Skin Ulcers in Papua New Guinea

Main results

- A single oral dose of azithromycin is efficacious for the treatment of *H. ducreyi* CU

- After treatment with single oral dose of azithromycin, 95% of *H. ducreyi* CU heal within 2 weeks.
- None of the non-healed *H. ducreyi* CU had detectable *H. ducreyi* DNA at follow up.
- *H. ducreyi* CU presents different clinical characteristics than yaws, yet definite clinical diagnosis requires molecular confirmation.

Conclusions

- *H. ducreyi* CU can now be co-targeted with yaws through yaws MDA campaigns using single dose oral azithormycin.
- MDA campaigns for *H. ducreyi* should be considered together with hygiene promotion measures to eliminate the skin reservoirs.
- Persistence of *H. ducreyi* CU after MDA with azithromycin is unlikely to be related to lack of response to the antibiotic treatment.

Limitations

- There was no untreated control group that would have provided stronger evidence of effect, but we can reasonably assume that the healing observed is attributable to treatment with an antibiotic agent.
- Because *H. ducreyi* has been found colonizing asymptomatic skin implies that the positivity of PCR may not, in itself, be sufficient to make a definitive diagnosis of *H. ducreyi* infection.

4.1.4 Study IV

Effectiveness of single-dose azithromycin to treat latent yaws: a longitudinal comparative cohort study

Main results

- Serological cure at 24 months was achieved in over 90% of patients after treatment with azithromycin, similar to those patients with active yaws.
- Successful treatment usually results in three or four dilutions decreases.
- Seroreversion may occur in less than 50% of cases.

Conclusions

- Single dose azithromycin is effective to treat latent yaws.
- Serological cure changes might take up to 12 months after treatment to be seen, thus earlier positive testing should not be considered as treatment failure.
- Non-treponemal tests can detect low titres long after treatment, thus this does not indicate unsuccessfully treated disease.
- Due to potential disease relapse months and even years after infection, treatment of latent cases is crucial for the elimination of the disease.

Limitations

- RPR test is read by naked eye and there is a degree of inter-observer variability in interpretation of test results that can lead to variation in RPR titre.
- Because of the observational nature of our study design, there was no untreated control group.
- By excluding people with baseline RPR titres of 1:2 or 1:4, the sample of participants with latent yaws might be biased in favour of early latent infection or people between the primary and secondary stages, which means that our cure rates cannot be generalized to patients with late stage- or low titer- disease.

4.1.5 Study V

Multiple class I and class II Haemophilus ducreyi strains cause cutaneous ulcers in children on an endemic island

Main results

- Class I and Class II *H. ducreyi* CU strains circulate in Lihir Island.
- Six different types (four class I and two class II) of *H. ducreyi* strains have been found circulating on Lihir island.
- Co-infection with both classes is common (23% of the cases). Pre-MDA strains continued circulating after MDA, and new strains were identified post-MDA (possibly related to importation events from outside the island or just strains that had not been initially detected because of the smaller sample size studied).

Conclusions

- MDA exerted no measurable changes on the composition of the *H. ducreyi* strains, suggesting that it is not more efficient in specific classes, types or strains.
- Single locus typing scheme using *dsrA* is sufficient to discriminate between CU strains.
- Factors other than treatment intervention (e.g. environmental reservoirs) must be addressed to achieve elimination of this infection.

Limitations

- Selective pressure could have potentially occurred in genes other than the one analysed in this study.
- Unambiguous *dsrA* sequences were found in only 66% of the 117 *H. ducreyi* positive samples. Repeated freeze thawing of the residual samples likely limited our ability to recover high quality sequences.
- The negative results yielded in our selective pressure analyses can be attributed to several reasons: biannual treatment with azithromycin of patients with CU results in very limited pressure on the organism; 2 years is a short time frame for selective pressure to become evident in a slowly evolving organism such as *H. ducreyi;* our data set was relatively small for selective pressure analyses.
- Given the mechanism of action of azithromycin, selective pressure by azithromycin may not be exerted on the *dsrA* gene, whose gene product confers serum resistance and binding to fibrinogen, vitronectin, and fibronectin to the organism.

4.1.6 Study VI

Haemophilus ducreyi DNA is detectable on the skin of asymptomatic children, flies and fomites in villages of Papua New Guinea

Main results

- *H. ducreyi* colonized the skin of 20% of healthy individuals. The proportion was similar in people that had or had not been in contact with an *H. ducreyi* CU patient.

- We did not find any evidence of skin colonization with *T. p. pertenue* which is consistent with the fragility of the bacteria to survive in the environment.
- *H. ducreyi* and *T. p. pertenue* DNA has been detected in flies and fomites of endemic settings.
- The ubiquity of *H. ducreyi* on the environment implies that individuals are at risk of infection even if there are no infected cases on their households.

Conclusions

- The presence of *H. ducreyi* in the skin of healthy subjects and environmental reservoirs such as linens may partially explain why MDA failed to reduce the proportion of *H. ducreyi* CU in Lihir island.
- The extent of the biological reservoir of *H. ducreyi* remains unknown.

Limitations

- Due to the fact that PCR test can detect non-viable bacteria or contaminating DNA, a swab positive for DNA does not give definitive evidence of colonization. Therefore further studies using culture systems are required to confirm our findings.
- Our study was significantly limited in its ability to assess asymptomatic carriage of *T. p. pertenue*, because the mass azithromycin treatment conducted earlier in these communities was so effective in reducing the prevalence of yaws ulcers.

4.1.7 Study VII

Re-emergence of yaws after single mass azithromycin treatment followed by targeted treatment: a longitudinal study

Main results

- Mass azithromycin treatment (coverage rate of 84%) followed by targeted treatment programs reduced the prevalence of active yaws from 1·8% to a minimum of 0·1% at 18 months.
- Infection began to re-emerge after 2 years with a significant increase to 0·4% at 42 months.
- After a major decline in *T. p. pertenue* strain gene diversity rebound was related to one strain type.

- The prevalence of high titre latent yaws in asymptomatic children aged 1–5 years fell from 13·7% before mass treatment to 1·5% at 24 months, and 0 cases at 42 months.
- At months 36 and 42, a total of five cases of active yaws, all from the same village, demonstrated clinical failure following azithromycin treatment and revealed mutations in the 23S ribosomal RNA genes conferring resistance to azithromycin.

Conclusions

- A single round of MDA is not sufficient to eliminate yaws in high endemic areas and therefore adjustments to the current strategy are required, for example multiple rounds of MDA.
- Strict surveillance after MDA implementation will be needed in the future to avoid resurgence of the disease and for the early identification of resistant cases.
- Antibiotic pressure could result in the selection of azithromycin resistant Treponema strains.

Limitations

- The study was initially designed to measure serologically-confirmed yaws, but on the light of serology deficiencies we moved to PCR testing; unfortunately we could calculate only an estimate of PCR-confirmed ulcers at baseline since only a subset of ulcers identified had a swab specimen collected.

4.2 Discussion

Between 1952 and 1964, WHO and the United Nations Children's Fund (UNICEF) led a multinational worldwide campaign to control and eventually eradicate yaws through mass treatment of active yaws cases with long acting injectable penicillin. The program reduced the number of cases of the disease by 95% worldwide, but it did not succeed to achieve eradication. Our findings in **Study I** (39) show that yaws has resurged and is currently endemic in several countries, particularly in the West and Central Africa region, Southeast Asia region, and the Pacific Islands region with over 80 million people living at risk of contracting the disease. The finding in 2012 that a single oral dose of azithromycin was as effective as parenteral penicillin in the treatment of yaws (35) has reignited the interest in CUs in the global public health research agenda, and has prompted WHO to revisit the worldwide eradication of the disease, and so in an effort to eradicate yaws by 2020, WHO has recently launched a new initiative, the Morges Strategy, employing mass azithromycin treatment of affected communities (2).

Traditionally, and due to lack of molecular confirmation tests in rural areas, all CUs have been clinically diagnosed as yaws, but recent research has shown that several pathogens can cause CU disease. Of particular importance has been the recent discovery of *H. ducreyi* as an agent of CU; our data in **Study II** (41) concludes that in the past 20 years there has been a reduction of genital-*H. ducreyi* infections in adults as a result of the implementation of syndromic management for genital ulcer disease in the 1950's, while *H. ducreyi*-CU in children seems to be highly prevalent and a matter of public health concern. Our results suggested that CU could be affecting mostly children in remote communities of the South Pacific and West Africa. Data that was made available after the completion of our **Study II** confirm that *H. ducreyi* was in fact responsible for a large proportion (up to a 60%) of all CUs (1,3,4,6).

Regarding programs piloting azithromycin MDA for yaws, demonstration projects have consistently shown a steep decrease in the prevalence of both yaws and *H. ducreyi* CU lesions but overall, they reported a less remarkable impact on the *H. ducreyi* CU compared to the profound decrease on the prevalence of yaws. We aimed to find a reason for the persistence of *H. ducreyi* infections in the communities after antibiotic mass treatment. Given the previous evidence that *H. ducreyi* genital ulcers respond well to azithromycin, and CU-*H ducreyi* strains from Vanuatu and Samoa were macrolide sensitive, a key question

was to test the efficacy of single dose azithromycin to treat *H. ducreyi* CU. Our results in **Study III** (42) provided definite clinical data on the efficacy of azithromycin to treat *H. ducrey*i CU (>95% healed). These results have implications for the treatment of individual patients and for the use of antibiotics in public health strategies to control CU in the tropics. Targeting *H. ducreyi* CU through yaws MDA campaigns should be considered together with hygiene promotion measures.

The 2012 study proved the effectiveness of azithromycin to treat active yaws (35), but as in venereal syphilis, yaws presents a latent disease stage where the bacteria are less metabolically active and less responsive to antibiotics. It is estimated that five to six people have latent yaws for each case of active yaws, and clinical relapse is possible for up to 5 years after infection. Treatment of both active and latent yaws is crucial for the success of an eradication programme. Entire community treatment allows individuals with latent disease to be exposed to curative treatment. With **Study IV** (43) we established the efficacy of single-dose azithromycin to treat latent yaws. These results support that azithromycin treatment failure is unlikely to play a part in persistent transmission of the disease after MDA.

So far, we had demonstrated that the persistence of *H. ducreyi* infections in the communities after MDA was not related to lack of effectiveness of azithromycin. We raised a question on the possibility that the bacteria may exist in a natural reservoir. In studies **V** and **VI** (44,45) we conducted studies to understand the dynamics of transmission of *H. ducreyi* CU strains. In **Study V** we aimed to assess the resilience of the bacteria to the community intervention. First, we had to develop a cost efficient system to type samples, and so we examined which components of the multilocus typing system are required to discriminate between strains without the need of running high costly WGS. We concluded that a single locus typing scheme using *dsrA* is sufficient to discriminate among *H. ducreyi* CU strains. Then, we used *H. ducreyi* DNA positive samples from Lihir Island to compare the bacterial population structure over time. We found six different types of *H. ducreyi* strains circulating on Lihir island and selective pressure analysis indicate that azithromycin MDA exerted no changes in the composition of the *H. ducreyi* strains. Our findings in study **VI** showed the presence of *H. ducreyi* DNA on the skin of asymptomatic villagers, flies and fomites. Although direct evidence of transmission is lacking, if *H. ducreyi* was able to remain in the environment through colonisation, this could explain the continued presence of this infection after MDA,

furthermore, if azithromycin does not reach the outer surface of the skin, it may not interrupt colonization, and these sources may perpetuate the infection in the community. Our data also suggests that environmental reservoirs must be addressed to achieve elimination of *H. ducreyi* CU. Given the prolonged prophylactic effect of azithromycin against experimental *H. ducreyi* infection, it is plausible that repeated mass treatment can confer a prophylactic effect to the population for long enough to clear the asymptomatic reservoir (46).

The current WHO recommendation for yaws eradication is initial MDA to the entire population with a single dose of oral azithromycin, followed by resurveys every 6 months in a targeted treatment program to detect and treat newly identified persons with active yaws and their contacts. Our team has been pioneer in the pilot implementation of a longitudinal study to assess the WHO strategy. In **Study VII** (47), we present data after following up a large cohort of 16,000 residents for 42 months. We concluded that a single round of MDA successfully reduced the prevalence of yaws clinical manifestations by 90% (35) but did not eliminate yaws in a highly endemic island community. The relapse of untreated latent infections was the most important factor that hindered elimination efforts in this community, along with, to a lesser extent, the reintroduction of yaws through cases of in-migration. We observed an increase of active yaws starting 2 years after the initial intervention.

During the last active case search round, we identified five azithromycin-resistant cases. Because these macrolide-resistant mutations appear to cause no fitness disadvantage in *T pallidum*, resistant strains would be likely to persist in communities even in the absence of antibiotic pressure. Therefore, communities where significant resistance is identified will need guidelines for clinical and operational management of macrolide-resistant yaws with Benzathine benzylpenicillin treatment to achieve cure and to avoid dissemination of resistant strains. Our findings have substantial implications for the scalability of yaws eradication programs internationally and support the following adaptations to the WHO strategy. First, a considerable effort to achieve coverage rates higher than 90% should be the goal in the first round of treatment. Second, distribution of a second or third round of azithromycin at 6–12 month intervals might be of substantial benefit. Third, efforts to eradicate yaws should aim to treat much broader geographical areas, especially in regions with substantial migration. Finally, clinical and biological surveillance needs to immediately detect drug resistance through the strengthening of capacities of laboratory networks in endemic countries.

4.3 Final Remarks

The scarcity of political will to improve the resources allocated to NTDs, inadequate funding to conduct community-based programs, and weaknesses in primary health-care systems in affected countries have hindered the reduction of the burden of CU-disease in the past two decades. However, despite the neglected condition of yaws, and all CU-diseases in general, renewed efforts are now being implemented in several endemic countries, and in the past 5 years a great deal of new information on CU-disease epidemiology and aetiology has been unveiled.

The main lessons learned from this experience are that yaws can be eliminated with sustained efforts; however, political and donor commitment need to be sustained until transmission is interrupted and health services must provide adequate outreach activities to remote communities in order to reach eradication of the disease and prevent re-emergence. It is important to emphasize that elimination of the disease from one geographical area is not guarantee against its re-introduction.

New strategies to control *H. ducreyi* along with yaws need to be explored. Syndromic care for ulcers using azithromycin and multiple rounds of MDA could be included in future interventions of the Morges Strategy for yaws eradication. Mathematical modelling has shown the value of multiple rounds of MDA to reduce *T. p. pertenue* infection, and this could, in parallel, reduce *H. ducreyi* infection and potentially skin carriage. In addition, skin hygiene and effective wound management using non-adherent dressings must be emphasized; given the potential carriage of *H. ducreyi* and *T. p. pertenue* by flies, covering ulcers may also help to prevent transmission.

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