

Optimising the use of molecular tools for the diagnosis of yaws

Morgan Munson^{a,†}, Benjamin Creswell^{a,†}, Kofi Kondobala^b, Bawa Ganiwu^b, Rita Dede Lomotey^b, Paul Oppong^b, Frederick Opoku Agyeman^b, Nana Kotye^b, Mukaila Diwura^b, Ebenezer Padi Ako^b, Shirley Victoria Simpson^c, Kennedy Kwasi Addo^c, Harry Pickering^a, Becca L. Handley^a, Joanna Houghton^a, Cynthia Kwakye^b and Michael Marks^{a,d,*}

^aClinical Research Department, Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, Keppel Street, London, UK; ^bGhana Health Service, Accra, Ghana; ^cDepartment of Bacteriology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana; ^dHospital for Tropical Diseases, Mortimer Market Centre, London, UK

*Corresponding author: Tel: +447984643424; E-mail: Michael.marks@lshtm.ac.uk

[†]These authors contributed equally to this work

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Background: Yaws is a neglected tropical disease and results in lesions of skin, soft tissues and bones. PCR plays an important part in surveillance.

Methods: Children suspected to have yaws were enrolled. From the largest lesion, paired swabs were collected, one in transport medium and one as a dry swab. In children with multiple lesions we collected additional swabs from up to four subsequent lesions. Swabs in transport medium were maintained in a cold chain while dry swabs were stored at ambient temperature. Swabs were tested by PCR for *Treponema pallidum* and *Haemophilus ducreyi*.

Results: Of 55 individuals, 10 (18%) had at least one positive PCR for *T. pallidum* and 12 (22%) had at least one positive result for *H. ducreyi*. Concordance was 100% between swabs in transport medium and dry swabs. One patient had PCR-confirmed yaws on the swab of a third lesion when both the first and second lesions were PCR-negative.

Conclusions: Storing swabs in transport medium and transporting in a cold chain did not improve yield, however, detection of *T. pallidum* is increased by swabbing additional lesions. As the target for yaws is eradication, approaches to sample collection need revisiting to ensure cases are not missed.

Keywords: Eradication, *Haemophilus ducreyi*, neglected tropical diseases, PCR, *Treponema pallidum* subspecies *pertenue*, Yaws

Introduction

Yaws, caused by the *Treponema pallidum* sp. *pertenue*, is a neglected tropical disease and results in lesions of the skin, soft tissues and bones.^{1,2} The disease predominantly affects children and is known to be endemic in 15 countries.³ Transmission of yaws occurs from person to person through direct skin contact with infectious exudates of early skin lesions of infected individuals.⁴ Early, active yaws is characterised by papillomatous and ulcerative lesions, which are highly infectious. Untreated lesions of early yaws may spontaneously heal but periods of relapse to infectious yaws may continue for a number of years. In a proportion of untreated individuals, the disease progresses

to highly destructive lesions of the skin and bones, which results in chronic disfigurement and disability.^{2,5}

Interest in yaws has increased in recent years, following studies showing azithromycin to be highly effective in the treatment of yaws, and that community mass treatment by azithromycin reduces the prevalence of both active and latent disease,^{6–10} culminating in renewed efforts by WHO to achieve global yaws eradication.¹¹ Despite global eradication efforts led by WHO and UNICEF between 1952 and 1965 and further national efforts between 1981 and 1983, Ghana remains endemic for yaws, and reports the most cases of any country in Africa.^{3,4} Yaws remains an important public health problem in Ghana and is

reported in all 10 regions and nearly all districts in the country. In 2008, Ghana launched the National Yaws Eradication Program (NYEP). The programme has succeeded in increasing the number of contacts treated per yaws case from 3.5 in 2008 to 10.8 in 2013¹² and in conducting pilot implementations of WHO's 'Morges' strategy for yaws eradication.^{10,11} Currently in Ghana, as in other endemic countries, the diagnosis of yaws is most often based on clinical grounds alone without the aid of serological or molecular investigations. Studies in many settings have indicated that clinical diagnosis alone may be unreliable^{13–15} and WHO has strongly advocated the introduction of diagnostic tests to improve the quality of epidemiological data on yaws in endemic countries.¹⁶

Serological methods remain the mainstay for laboratory confirmation of yaws and include both treponemal and non-treponemal tests. Treponemal tests such as the *Treponema pallidum* particle agglutination assay are highly specific, but remain positive for life following infection. Diagnosis of current disease therefore relies on both a positive treponemal test and also a reactive non-treponemal test such as the rapid plasma regain or the Venereal Disease Research Laboratory test. Titres of non-treponemal tests rise during infection and fall following successful treatment.² Rapid diagnostic tests that combine treponemal and non-treponemal components, such as the dual path platform (DPP) 'syphilis screen and confirm' assay, have now been validated for the diagnosis of yaws and have largely replaced traditional laboratory assays within the programmatic setting.^{17–20}

PCR methods to detect treponemal DNA from genital ulcers of venereal syphilis have been available for more than two decades.^{21,22} More PCR assays have been developed that are able to differentiate between the DNA of different subspecies of *T. pallidum* complex.^{14,23} Due to primer binding site mutations occurring in some strains of yaws, the current recommendation is to use a pan-treponemal PCR target such as *polA* or *tp47* for the diagnosis of yaws.²⁴ When PCR is used to assess individuals with clinical and serological evidence of yaws a large proportion of lesions are found to contain no treponemal DNA.^{23,25} In several studies, *Haemophilus ducreyi* has been demonstrated to be a common cause of skin lesions, which may be confused with those of yaws in endemic countries in the South Pacific islands and Africa.^{15,25,26} For this reason there is a recognition that PCR will play an increasingly important part in yaws surveillance efforts worldwide.¹⁶

Currently, there are limited data on the optimal approach to collecting samples for molecular confirmation of yaws. In a number of studies, samples have been collected from a single lesion per individual, placed in transport medium and shipped within a strict cold chain.^{23,25,27} A number of studies assessing the use of PCR for the diagnosis of yaws have suggested that transport medium is unnecessary and that dry swabs perform equally well.²⁸ A move to dry swabs and shipping at ambient temperatures would reduce programmatic costs and potentially increase the feasibility of incorporating molecular testing into the programmatic context. In this study we aimed to evaluate whether the collection of additional swabs, from more than a single lesion, and the presence or absence of transport medium, affected the performance of PCR for the diagnosis of yaws.

Methods

Case finding and screening

The study was conducted in the West Akim and Upper West Akim districts in the eastern region of Ghana. These districts were selected as they are known to be highly endemic for yaws. We conducted active case finding searches in July 2018 in collaboration with NYEP. Field workers visited schools to identify children with lesions consistent with yaws. Children with sores, ulcers or skin lesions were asked to come forward for a more detailed examination as per routine NYEP protocol during case searching. Children with yaws-like ulcers were first screened with the SD Bioline syphilis test (Abbott, Chicago, Illinois, USA) and those who tested positive were then tested with the DPP syphilis screen and confirm test (Chembio, Medford, New York, USA). Individuals with a dual positive (treponemal and non-treponemal) DPP assay were enrolled into the study.

Swab collection

We identified a maximum of five yaws-like lesions per individual prior to treatment. Swabs were taken in line with WHO recommendations for swabbing yaws lesions. In brief, a single Dacron swab was used to obtain tissue from each lesion. This was performed by pressing the head of the swab into the centre of the lesion and rotating the swab over the area. If a lesion or papilloma was dry, the area was moistened using sterile saline on some gauze and the outermost epithelial lining was gently removed using a blunt object before swabbing. We identified the largest, most exudative lesions first. From this lesion, paired swabs were collected, the first in transport medium and the second as a dry swab. From a maximum of four subsequent lesions we collected additional dry swabs at baseline. All individuals were then offered a single dose of azithromycin in line with national guidelines. Follow-up dry swabs were collected in the same manner from the first three lesions at 8, 24, 36 and 48 h after treatment with azithromycin.

Swab storage

Swabs in transport medium were placed immediately into a cool box and were frozen at -20°C at the laboratories of the Noguchi Memorial Institute for Medical Research. Swabs in transport medium were then shipped on dry ice to the UK, where they were again frozen at -20°C until testing. Dry swabs not in transport medium were stored at ambient temperature in the field for the duration of the study and shipped at ambient temperature to the UK for testing.

Swab testing

DNA was extracted from lesion swabs using the QIAamp DNA Mini Kit according to the manufacturer's instructions. Multiplex PCR targeting the *tp47* of *T. pallidum* and the *hhdA* gene of *H. ducreyi* was performed on a Rotorgene 3000 as previously described.²² If baseline swabs were PCR-positive for either target, the follow-up swabs from the same lesion were extracted and tested using the same PCR targets. Positive and no-template controls were included in every PCR run.

Analysis

We compared the proportion of samples positive by PCR for either *T. pallidum* or *H. ducreyi* for the paired lesion samples collected from the 'target' lesion. We considered the samples collected in transport medium and maintained in cold chain as the gold standard and the dry swabs stored at ambient temperature to be the comparator. We calculated the proportion of individuals diagnosed with either *T. pallidum* or *H. ducreyi* based only on a swab of the target lesion to the proportion diagnosed based on swabbing of multiple lesions. For individuals whose lesions were PCR-positive at baseline, we assessed the number of lesions in which pathogen DNA remained detectable after treatment with azithromycin.

Results

Approximately 3000 children were screened during active case searches to identify 55 individuals with clinical evidence of yaws and a dual positive DPP assay. The median age of individuals enrolled in the study was 10 y (IQR 9–12) and a majority of patients were male ($n=33$, 60%). For dry swabs the median duration of storage at ambient temperature was 22 d (IQR 21–32).

Overall, 10 (18%) patients had at least one positive PCR result for *T. pallidum* and 12 (22%) patients had at least one positive PCR result for *H. ducreyi*, representing 22% of all participants. Of the 55 children included in this study, 53 had a swab from their target lesion collected both in transport medium and a dry swab at baseline. There was 100% concordance between the results of PCR conducted on swabs collected in transport medium and maintained in a cold chain and dry swabs stored at ambient temperature.

Overall, 111 baseline swabs were collected from the 55 patients enrolled in the study. The median number of swabs per patient was two (IQR 1–2.5). Overall, a total of 14 swabs (12.6%) were positive for *T. pallidum*, of which nine swabs were from target lesions. Four swabs were positive from additional lesions of patients whose target lesion was also PCR-positive for *T. pallidum*. One patient had PCR-confirmed yaws diagnosed based on the swab of a third lesion when both the target and secondary swabbed lesion were PCR-negative. Overall, a total of 17 swabs (15.3%) were positive for *H. ducreyi*, of which nine were from target lesions. Five swabs were positive from additional lesions of patients whose target lesions were also PCR-positive for *H. ducreyi* while three samples were positive from individuals whose target lesions were PCR-negative for *H. ducreyi*. Of 14 swabs that were positive at baseline for *T. pallidum*, follow-up swabs were available for 11 lesions. Of these 11 lesions, every available follow-up swab remained PCR-positive, including the seven patients who had swabs collected 48 h after treatment.

Discussion

PCR has become an important tool in the diagnosis and surveillance for yaws as part of global eradication efforts. In this study we demonstrate that storing swabs in transport medium and transporting them within a cold chain does not improve the diagnostic yield compared with dry swabs stored at ambient temperature. Our findings may have significant cost

and logistical implications for programmes and should increase the feasibility of incorporating molecular diagnostics into routine care and surveillance activities. In addition, despite our relatively small sample size, we also demonstrated that the detection of *T. pallidum* could be increased by swabbing additional lesions beyond the target lesion. Swabbing only a single lesion has been standard practice in all studies to date^{15,23,25,27} but our data suggest that such an approach will result in misclassification of cases. As the target for yaws is global eradication, detection of every case is vital and our study demonstrates that the approach to sample collection will need to be revisited to ensure cases of active yaws are not missed.

Access to PCR to confirm the diagnosis of yaws remains extremely limited in all endemic countries outside of research studies. Diagnostic confusion with *H. ducreyi* and the need to monitor for the emergence of azithromycin resistance in *T. pallidum* highlight that molecular tools will be required to support global yaws eradication efforts.^{16,29} This is the first study to assess the impact of the use of transport medium and a cold chain on the diagnostic yield of lesion swabs for PCR. The cycle threshold value of the PCR assay was also similar for both dry swabs and swabs collected in transport medium (data not shown) suggesting that DNA was also not adversely affected by the absence of transport medium or a cold chain. Taken together with existing studies on syphilis, our data suggest that there is no benefit in using transport medium or a cold chain for routine diagnostic purposes. Simplifying sample collection, storage and transport requirements should reduce costs and improve the feasibility of implementing PCR as part of programmatic activities.

Many patients with yaws have multiple lesions on examination, however, to date, studies have focused on collecting a sample from only the largest target lesion. Such a strategy presumes that all skin lesions in an individual are caused by the same pathogen. In this study, we clearly demonstrate that this may result in missing PCR-positive cases of yaws. As the aim of the WHO strategy is global eradication, detection of every case is vital and our data strongly argues that consideration be given to swabbing multiple skin lesions where these are present to ensure all cases of active yaws are detected. We used separate swabs for each lesion and tested these separately. Pooling of samples for testing or use of a single swab for multiple lesions may facilitate similar increases in diagnostic yield while avoiding the additional costs of performing separate PCR on multiple lesions. We also demonstrated that lesions remain positive by PCR up to 48 h after treatment. While a positive PCR after treatment might simply represent detection of DNA and not viable organisms, our findings support the WHO recommendation to provide wound dressings as well as treatment to reduce any potential risk of ongoing transmission post-treatment.

One major limitation of our study is the relatively small sample size. It is possible that with a larger number of *T. pallidum* PCR-positive lesions, we might have been able to demonstrate a difference in the performance of PCR between samples collected in transport medium compared with dry swabs. However, studies in syphilis have also demonstrated no difference between samples collected in transport medium or dry swabs.^{28,30,31} Samples were stored at ambient temperature for a median of more than 20 d, which is likely to be longer than the average duration of sample transport under programmatic conditions. We believe,

therefore, that our results are robust and that in routine settings there is no requirement for transport medium or a cold chain when collecting diagnostic samples. Also, swabbing of additional lesions detected only a single extra case of PCR-confirmed active yaws. However, this additional case represented 10% of all cases of active yaws diagnosed in the study. For the global eradication campaign, it will be vital to detect every case, and our data strongly support the need to consider swabbing multiple lesions when they are present.

Conclusions

Molecular tests play a central role in surveillance and monitoring during yaws eradication efforts but there have only been a few studies evaluating how best to operationalise this tool. By simplifying sample collection, storage and transport, and highlighting the importance of sampling multiple lesions, the data from our study should enable programmes to optimise their use of this critical diagnostic tool and support successful global eradication efforts.

Supplementary data

Supplementary data are available at Transactions online (<http://trstmh.oxfordjournals.org/>).

Authors' contributions: MM, BC, JH, CK and MM designed the study. MM, BC, HP, RH, JH, CK and MM analysed and interpreted the data. MM, BC, CK and MM prepared the first draft of the manuscript. All authors contributed to study implementation and revision of the manuscript.

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Ethical approval: Ethical approval for the study was obtained from the Ghana Health Services Ethics Review Committee and the London School of Hygiene and Tropical Medicine. Parents or guardians provided written informed consent for children to participate in the study and, where appropriate, assent was also obtained from children.

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