Treponema pallidum detection in lesion and non-lesion sites in men who have sex with men with early syphilis: a prospective, cross-sectional study

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Summary

Background Syphilis transmission is increasing, and precisely how *Treponema pallidum* is transmitted sexually from person to person is unclear. We aimed to determine the frequency of *T pallidum* shedding from potentially asymptomatic sites and the stage of infection at which shedding is most frequent in men who have sex with men (MSM), who have been disproportionately affected by syphilis.

Methods We did a prospective, cross-sectional study in MSM recruited from Melbourne Sexual Health Centre (Melbourne, VIC, Australia). Men were eligible if they were aged 18 years or older, reported sex with men during the past 12 months, and had laboratory confirmed primary, secondary, or early latent syphilis, consistent with Australian definitions. Primary and secondary syphilis lesions were swabbed and non-lesion samples were collected via oral rinse, oral cavity swab, anal canal swab, urine, and semen. Samples were tested for *T pallidum* using PCR assays targeting *polA* (lesion and non-lesion samples) and 47 kDa (non-lesion samples only) gene targets. The primary outcome was the proportion of men with *T pallidum* detected from potentially asymptomatic sites—namely, the mouth, anus, urethra, and semen.

Findings Between Nov 30, 2015, and May 23, 2019, 246 MSM were screened for inclusion, of whom 200 had serologically confirmed early syphilis and were included in the study: 54 (27%) of 200 had primary syphilis, 93 (47%) had secondary syphilis, and 53 (27%) had early latent syphilis. *T pallidum* DNA was detected in 48 (24%; 95% CI 18·3–30·5) of 200 men by oral rinse or oral lesion swab, or both, of whom 24 had no oral lesions. Oral *T pallidum* detection was most frequent in those with secondary syphilis compared with those at other stages of disease (41 [44%] of 93 *vs* seven [7%] of 107; p<0·0001), and in men with rapid plasma reagin titres of 1/64 or higher compared with those with lower titres (37 [32%] of 117 *vs* 11 [13%] of 83; p=0·0026). *T pallidum* was detected by anal canal swab or anal lesion swab, or both, in 45 (23·0%; 95% CI 17·3–29·5) of 196 men with available samples, of whom ten had no anal lesion. Furthermore, *T pallidum* was detected in urine samples of 12 (6·1%, 3·2–10·3) of 198 men and in semen samples from six (12·0%, 4·5–24·3) of 50 men who provided samples. Among the 93 men with secondary syphilis, 69 (74%) had *T pallidum* detected at any site, and 24 (26%) had detection at two or more separate sites. Among the 54 men with primary syphilis, 49 (91%) had *T pallidum* detected at any site, and 11 (20%) had detection at two or more separate sites. Among the 53 men with early latent syphilis, four (8%) had *T pallidum* detected at any site and none had *T pallidum* detected at two or more separate sites.

Interpretation Unrecognised oral and anal shedding of *T pallidum* occurs in MSM with early syphilis, most frequently in those with secondary syphilis, suggesting secondary syphilis is the most infectious stage and that earlier detection and treatment of syphilis to prevent progression to the secondary stage might improve syphilis control. Future research is needed to ascertain the contribution of shedding of *T pallidum* from non-lesion sites to transmission of syphilis.

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Introduction

Syphilis remains a global health problem with an estimated 6 million new infections worldwide in 2016.[1] Men who have sex with men (MSM) comprise the largest proportion of cases of syphilis.[2] Data from a study of high-income countries indicated the proportion of syphilis cases in MSM increased from 26·8% to 55·0% between 2000 and 2013.[3] In the past 5 years, syphilis has re-emerged among heterosexual people in high-income countries, including the USA, Japan, and Australia.[4] Syphilis results in serious morbidity, including neurosyphilis, ocular syphilis, and congenital syphilis, if untreated.

Although transmission of syphilis is increasing, precisely how *Treponema pallidum* is transmitted sexually from person to person is unclear. *T pallidum* is widely assumed to be transmitted from overt mucocutaneous

Research in context

Evidence before this study

We searched PubMed between Jan 1, 2000, and June 3, 2020, for English-language articles using the terms ("oral" OR "anal") AND "*Treponema pallidum*" in the title or abstract, or both. We identified 145 studies, one of which aimed to determine the frequency of *Treponema pallidum* detection in the oral cavity. In this study of mainly HIV-positive men who have sex with men (MSM), different oral sampling methods were used depending on whether or not oral lesions were present. This study did not include anal sampling of all men for *T pallidum*. We did not identify any studies that systematically sampled the oral cavity for *T pallidum* testing by PCR in cohorts that were mostly HIV negative, or that compared *T pallidum* detection from lesions with potentially asymptomatic sites. We aimed to determine the frequency of *T pallidum* shedding from asymptomatic sites (eg, the oral cavity and anus) compared with lesions, and the stage of syphilis with the most frequent shedding, in MSM with early syphilis.

Added value of this study

To our knowledge, this study is the first in which lesion and non-lesion sites have been systematically sampled for *T pallidum* in both HIV-positive and HIV-negative MSM across all stages of early syphilis infection (primary, secondary, and early latent). We found oral and anal shedding of *T pallidum* was most frequent during secondary syphilis, often in the absence of overt syphilis lesions, and that this shedding occurred in HIV-positive and HIV-negative men.

Implications of all the available evidence

Oral and anal shedding of *T pallidum* might be factors in sustaining the transmission of syphilis, although studies are needed to demonstrate *T pallidum* viability from these sites. If the bacteria are viable, our findings suggest that secondary syphilis is the most infectious stage and that public health interventions that consider earlier detection of syphilis to prevent progression to the secondary stage might improve control.

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lesions in those with primary and secondary syphilis. However, transmission might also occur from other sites such as the mouth, genitals, and anus, where lesions might be occult or where *T pallidum* might be shed asymptomatically. In one study of MSM with syphilis of all stages, most of whom were HIV positive, 42% of men had *T pallidum* detected orally.[5] To date, few studies have compared the frequency at which *T pallidum* is shed from syphilis lesions compared with other potentially asymptomatic sites.[5,6] Moreover, how infectious the different stages of syphilis are relative to each other is poorly understood.

Our primary aim in this study was to determine the frequency of *T pallidum* shedding from potentially asymptomatic sites—namely, the mouth, anus, urethra, and semen—in MSM with early syphilis. Our secondary aim was to determine the stage of syphilis with the most frequent shedding from these sites.

Methods

Study design and population

In this prospective cross-sectional study in MSM with untreated early syphilis, participants were recruited from Melbourne Sexual Health Centre, the major publicly funded sexually transmitted infection clinic in Victoria, Australia. This clinic provides services to the state of Victoria with approximately 50 000 attendances per year. Men were eligible if they were aged 18 years or older, reported sex with men during the past 12 months, and had laboratory confirmed primary, secondary, or early latent syphilis, consistent with Australian definitions.[7,8] Men were excluded if syphilis was not confirmed by testing or if antibiotics were used within the past 1 month. Participants provided written informed consent, including separate consent for taking of and use of de-identified photographs (for uses including teaching, education, research or publication in journals, books or on websites), and self-completed a paper-based questionnaire with questions about their history of syphilis, HIV testing and treatment, and sexual history over the past 12 months. Ethical approval for this study was granted by the Alfred Hospital Ethics Committee (number 487/15).

Procedures and definitions

A targeted medical history was obtained via face-to-face consultation with investigators RW or JMT and the self-completed questionnaire, including symptoms, previous syphilis infection, and current use of HIV pre-exposure prophylaxis (PrEP). A comprehensive full-body examination was done to document the site, type, number, size, and distribution of any mucocutaneous syphilis lesions using proforma body charts. The anal examination was limited to external examination only and proctoscopy was not routinely done, unless clinically indicated. All aspects of the oral cavity were thoroughly examined under direct visualisation.

Descriptors for lesions were predefined. Ulcers of primary syphilis were described as chancres if indurated. Lesion referred to any oral, anal, perianal, or genital (penile or scrotal) mucocutaneous manifestation of primary or secondary syphilis. The term condylomata lata was used to describe raised, pale, moist lesions in secondary syphilis. Anal lesions were within the anal canal, rectal lesions were above the anal canal, and perianal lesions were external to the anal verge. The term rash was used to refer to any cutaneous manifestations seen in secondary syphilis, excluding oral, anal, and

genital lesions, including macular, papular, and nodular forms. Two sexual health physicians (JMT, ID) independently determined a participant's stage of syphilis with adjudication by a third physician (MYC) in case of disagreement. Staging as primary or secondary disease was consistent with US and Australian classifications, and early latent syphilis was staged according to the Australian classification—ie, less than 2 years duration.[7,8] For first syphilis infections, primary and secondary syphilis were defined on the basis of clinical signs together with laboratory confirmation, with detection of *T pallidum* or reactive serology, or both. Early latent syphilis was defined by reactive syphilis serology and seroconversion within the past 2 years together with the absence of syphilis lesions. Repeat syphilis was defined by a positive *T pallidum* PCR result or a four times or more increase in rapid plasma reagin (RPR) antibody titres after a previous syphilis infection.

T pallidum DNA was collected from primary or secondary lesions or rash using swabs without surface abrasion, henceforth referred to as lesion samples. All lesions were sampled using a FLOQSwab (Copan Diagnostics, Murrieta, CA, USA). If multiple lesions were present at one site, the largest or most developed lesion was sampled. The entire surface of the lesion was sampled once by pressing and rolling the swab over the lesion. Ulcerated and oral lesions were sampled with a dry swab and non-ulcerated lesions and rashes were sampled using a swab moistened with sterile water. Generalised secondary rashes were sampled over a 10 cm by 10 cm square of the most confluent area of rash.

See Online for appendix 1

	All cases (n=200)	Primary syphilis (n=54)	Secondary syphilis (n=93)	Early latent syphilis (n=53)
Age, years	31 (26–39)	30 (25–39)	31 (26–39)	33 (27–41)
HIV positive	62 (31%)	9 (17%)	28 (30%)	25 (47%)
CD4 count, cells per µL	611 (467–758)	709 (568–764)	494 (373–748)	648 (550–763)
On ART	55/62 (89%)	9/9 (100%)	23/28 (82%)	23/25 (92%)
On ART and viral load <50 copies per mL	53/55 (96%)	9/9 (100%)	22/23 (96%)	22/23 (96%)
HIV negative	138 (69%)	45 (83%)	65 (70%)	28 (53%)
Taking PrEP	29/138 (21%)	12/45 (27%)	9/65 (14%)	8/28 (29%)
Not taking PrEP	109/138 (79%)	33/45 (73%)	56/65 (86%)	20/28 (71%)
Reported contact with someone with syphilis	30 (15%)	7 (13%)	17 (18%)	6 (11%)
Self-reported sexual behaviour in past 12 months				
Number of sexual partners	10 (5–20)	15 (7–30)	9 (3–20)	10 (7–24)
Receptive penile-oral sex	190 (95%)	49 (91%)	90 (97%)	51 (96%)
Insertive penile-oral sex	194 (97%)	51 (94%)	91 (98%)	52 (98%)
Receptive penile-anal sex	169 (85%)	37 (69%)	84 (90%)	48 (91%)
Condomless receptive penile-anal sex	134 (67%)	27 (50%)	66 (71%)	41 (77%)
Insertive penile-anal sex	171 (86%)	50 (93%)	73 (78%)	48 (91%)
Condomless insertive penile-anal sex	140 (70%)	44 (82%)	60 (65%)	36 (68%)

Data are median (IQR), n (%), or n/N (%). ART=antiretroviral therapy. MSM=men who have sex with men. PrEP=pre-exposure prophylaxis.

Table 1: **Characteristics of MSM with early syphilis included in the study, by stage**

Non-lesion samples were also obtained from all men for *T pallidum* DNA detection whether local lesions were present or not. These samples were an oral rinse with 10 mL of sterile water swished and gargled for 15 s; a swab of the whole mucosal lining of the oral cavity; a blind anal canal swab, inserted 2 cm into the anal canal and rotated; and first pass urine. Because the optimal method for oral *T pallidum* detection was unknown, we used two different methods of oral sample collection and we did a prespecified interim analysis 2 years into the study to determine which method of oral sampling was superior.

After the collection of all study samples on the day of enrolment, all men were given antibiotic treatment for presumptive syphilis infection with either benzathine benzylpenicillin 1·8 g single dose by intramuscular injection or doxycycline 100 mg orally twice daily for 14 days. Participants were not able to supply a semen sample at the site, so all were given a take-home kit for collection and asked to collect a sample on the same day as the study visit and return it to the clinic via post on the same day, with AUD$30 reimbursement. Hence, semen samples were collected after syphilis treatment. Semen samples were received at the clinic a median of 6 days after the study visit.

T pallidum PCR testing of lesion samples was done using an in-house real-time PCR assay targeting the *polA* gene, with a cycle threshold (Ct) cutoff of 38, which was previously validated for clinical use with lesion samples.[9] Non-lesion samples were tested using the *polA* assay together with a second in-house PCR assay targeting the 47 kDa antigen gene to confirm they were true positive results. All men had serological testing for syphilis using a rapid plasma reagin test (Becton Dickinson, Franklin Lakes, NJ, USA) and a *T pallidum* particle agglutination assay (Fujirebio, Tokyo, Japan), and either a *T pallidum* ELISA immunoassay (Trepanostika EIA; BioMerieux, Marcy-l'Étoile, France) if tested before 2016, or a chemiluminescent immunoassay (LIAISON Treponema screen; DiaSorin, Saluggia, Italy) if tested from January, 2016, onwards. Serum samples were also tested for *T pallidum* IgM using the Euroimmun Anti-Treponema pallidum ELISA (IgM; Lubeck, Germany). Detailed laboratory methods are in appendix 1 (pp 1–2).

Men were screened for gonorrhoea and chlamydia using urine samples and swabs of the pharynx and anal canal using the Aptima Combo 2 Transcription-Mediated Amplification Assay (Hologic Gen-Probe, San Diego, CA, USA), and were given antibiotic treatment if positive. First line treatment for early syphilis consisted of benzathine benzylpenicillin 1·8 g single dose by intramuscular injection. Follow-up

serology was recommended 6 months after treatment. Men who were HIV negative were also screened for HIV, and any men with a new HIV diagnosis were referred for HIV management.

Outcomes

The primary outcome was the proportion of men with *T pallidum* detected from potentially asymptomatic sites—namely, the mouth, anus, urethra, and semen. The key secondary outcome was the proportion of men with *T pallidum* detected at these sites, according to the stage of syphilis. A further secondary outcome was the association between *T pallidum* detection from these sites and rapid plasma reagin titres.

Statistical analysis

For categorical variables, we calculated proportions and 95% CIs. For continuous variables, we calculated the median and IQR. We compared categorical variables, such as *T pallidum* detection by stage of infection and rapid plasma reagin titre, using Fisher's exact test. We used the Mann-Whitney *U* test to compare continuous variables, such as PCR Ct values, between assay types, between secondary versus primary lesions, and according to different rapid plasma reagin titres. We calculated exact binomial 95% CIs for the primary outcome using STATA version 14.

We calculated that we required a sample size of 200 men on the basis of 95% CIs around anticipated proportions of men with oral or anal *T pallidum* detection, with oral *T pallidum* being detected in 42% of MSM in a previous study.[5] The 95% CIs for anticipated site-specific proportions ranged from 10% (95% CI 6·4–15) to 40% (33–47) for oral and anal sites.[5]

The cutoffs for the analyses of rapid plasma reagin titre and *T pallidum* detection by site were exploratory and the titre cutoffs were not predetermined.

We considered p values of less than 0·05 to be statistically significant. We analysed all data using SPSS, version 25.0.0.0.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Nov 30, 2015, and May 23, 2019, 246 MSM were screened for inclusion, of whom 46 men with suspected syphilis were excluded because their serology or PCR results, or both, were negative for *T pallidum* or they had latent syphilis of unknown duration. Hence, 200 men with laboratory-confirmed early syphilis were included, all with positive results on the *T pallidum* particle agglutination assay and either the *T pallidum* ELISA immunoassay (n=3) or the chemiluminescent immunoassay (n=197). 138 (69%) men were HIV negative, of whom 29 (21%) were taking HIV PrEP (table 1). Among the 62 men who were HIV positive, including three diagnosed with HIV at enrolment, the median CD4 count was 611 (IQR 474–755) cells per µL. 55 (89%) of 62 men with HIV were taking antiretroviral therapy, of whom 53 (96%) had a HIV viral load of less than 50 copies per mL.

76 (38%) of 200 men had a repeat syphilis infection (table 2), which was more common among those who were HIV positive than those who were HIV negative (40 [65%] of 62 *vs* 36 [35%] of 138; p<0·0001). Overall, 200 oral rinse samples, 134 oral cavity swabs, 196 anal canal swabs, 198 urine samples, and 50 semen samples were collected. Proctoscopy was done for two participants because rectal symptoms were reported. The clinical and laboratory characteristics of men are summarised in table 2 with details for each participant in appendix 1 (pp 4–19). Photographs of syphilis lesions among 69 of

	All cases (n=200)	Primary syphilis (n=54)	Secondary syphilis (n=93)	Early latent syphilis (n=53)
First syphilis infection	124 (62%)	38 (70%)	66 (71%)	20 (38%)
Repeat syphilis infection	76 (38%)	16 (30%)	27 (29%)	33 (62%)
Neurosyphilis	2 (1%)	0	2 (2%)	0
Lesion samples				
Oral lesions present	34/200 (17%)	3/54 (6%)	31/93 (33%)	0/53
Swabbed and PCR positive	20/200 (10%)	2/54 (4%)	18/93 (19%)	0/53
Anal, perianal, or rectal lesion or lesions present	37/200 (19%)	14/54 (26%)	23/93 (25%)	0/53
Swabbed and PCR positive	34/200 (17%)	13/54 (24%)	21/93 (23%)	0/53
Penile or scrotal lesion or lesions present	88/200 (44%)	37/54 (69%)	51/93 (55%)	0/53
Swabbed and PCR positive	53/200 (27%)	34/54 (63%)	19/93 (20%)	0/53
Rash of secondary syphilis present	78/200 (39%)	0/54	78/93 (84%)	0/53
Swabbed and PCR positive	4/200 (2%)	0/54 (0%)	3/93 (3%)	0/53 (0%)
Any lesion site PCR positive	97/200 (49%)	49/54 (91%)	48/93 (52%)	0/53 (0%)
PCR positivity of non-lesion samples				
Oral rinse	40/200 (20%)	4/54 (7%)	34/93 (37%)	2/53 (4%)
Anal canal swab	30/196 (15%)	9/52 (17%)	19/92 (21%)	2/52 (4%)
Urine	12/198 (6%)	6/53 (11%)	6/93 (6%)	0/52 (0%)
Semen	6/50 (12%)	3/14 (21%)	3/20 (15%)	0/16 (0%)
Any lesion site or non-lesion sample PCR positive	122/200 (61%)	49/54 (91%)	69/93 (74%)	4/53 (8%)
Serology				
ELISA immunassay, CLIA, or TPPA reactive*	200/200 (100%)	54/54 (100%)	93/93 (100%)	53/53 (100%)
Rapid plasma reagin titre	1/64 (NR to 1/2048)	1/16 (NR to 1/1024)	1/128 (1/8 to 1/2048)	1/32 (1/1 to 1/1024)
ELISA immunoassay IgM reactive†	150/197 (76%)	38/52 (73%)	87/93 (94%)	25/52 (48%)

Data are n (%), n/N (%), or median (range). CLIA=chemiluminescent immunoassay. MSM=men who have sex with men. NA=not applicable. NR=not reactive. TPPA=*Treponema pallidum* particle agglutination assay. *All cases were positive on TPPA testing and ELISA immunoassay or CLIA. †Three participants did not have ELISA immunoassay IgM done.

Table 2: **Clinical characteristics, PCR test results, and laboratory characteristics of MSM with early syphilis, by stage**

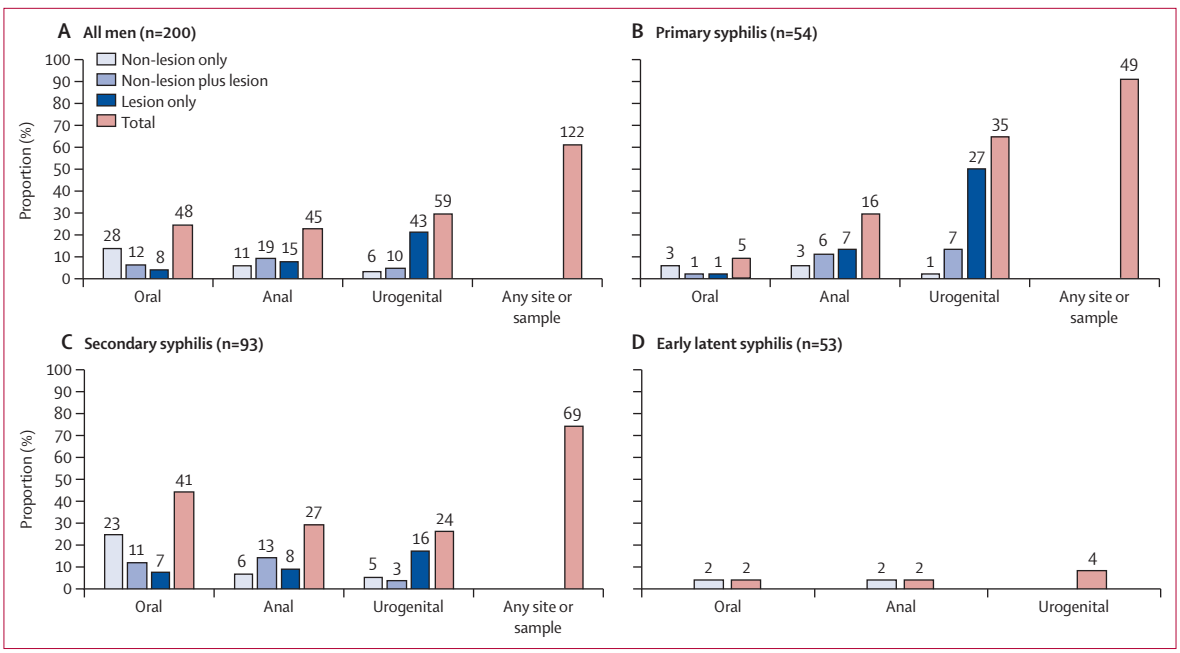

Figure 1: **Proportion of men with *Treponema pallidum* DNA detected by lesion swab or non-lesion sampling and total site detection, by stage of syphilis**
Numbers above each bar are number of men, and the height of the bars shows the proportion of the total population indicted in each graph title. For the type of detection, non-lesion only means only a non-lesion sample (oral rinse, anal canal swab, urine, or semen) was *T pallidum* PCR positive; non-lesion plus lesion means a non-lesion sample and a lesion swab from the same site were both *T pallidum* PCR positive; and lesion only means only a lesion swab was *T pallidum* PCR positive. For detection sites, the urogenital non-lesion category includes urine and semen results combined and total urogenital includes these samples plus genital lesions. Any site or sample is the proportion of men with at least one positive *T pallidum* PCR sample from any lesion or non-lesion sample. The total at any site or sample is the total number of men with *T pallidum* detection from any site and might not equal the sum of the other categories combined because some men had detection at multiple sites.

80 participants who consented to photography, and had pictures of sufficient quality for inclusion here, are shown in appendix 2.

See Online for appendix 2

54 (27%) men were diagnosed with primary syphilis, 93 (47%) with secondary syphilis, and 53 (27%) with early latent syphilis. One man with secondary syphilis had otosyphilis, and another also with secondary syphilis had ocular syphilis and otosyphilis. 53 (27%) men were circumcised. An interim analysis 2 years into the study found oral cavity swabs were positive in only four (15%) of 27 participants who had an oral rinse that was positive; therefore, oral cavity swab collection was subsequently ceased.

24·0% (95% CI 18·3–30·5; 48 of 200) of men tested positive for *T pallidum* in oral samples (lesion and non-lesion), of whom 24 had no oral lesions. 23·0% (17·3–29·5; 45 of 196) tested positive in samples from anal, perianal, or rectal lesions or the anal canal, of whom ten had no anal lesion. 6·1% (3·2–10·3; 12 of 198) tested positive in urine, and 12·0% (4·5–24·3; six of 50) tested positive in semen (table 2, figure 1). From the frequency of detection of *T pallidum* from lesion and non-lesion samples, we found that *T pallidum* shedding occurred more frequently from the mouth and anus during secondary syphilis than during primary or early latent syphilis (figure 1).

Detection of *T pallidum* by oral rinse or oral lesion swab, or both, was more frequent in those with secondary syphilis than in those with primary or early latent syphilis (detected in 41 [44%] of 93 with secondary syphilis *vs* seven [7%] of 107 with other stages of disease; p<0·0001). Detection of *T pallidum* by oral rinse or oral lesion swab, or both, was less frequent in those with primary syphilis than in those with secondary or early latent syphilis (detected in five [9%] of 54 with primary syphilis *vs* 43 [30%] of 146 with other stages of disease; p<0·0026). Detection of *T pallidum* by oral rinse or oral lesion swab, or both, was less frequent in those with early latent syphilis than in those with primary or secondary syphilis (detected in two [4%] of 53 with early latent syphilis *vs* 46 [31%] of 147 with other stages of disease; p<0·0001). Oral detection of *T pallidum* was also more common in men with a rapid plasma reagin titre of 1/64 or higher than in those with lower titres (37 [32%] of 117 *vs* 11 [13%] of 83; p=0·0026). We found no significant difference in detection of *T pallidum* in the anus in participants with secondary syphilis who provided these samples compared with those at other stages of disease (27 [29%] of 92 with secondary syphilis *vs* 18 [17%] of 104 at other stages; p=0·061). We found no significant difference in detection of *T pallidum* in the anus in participants with primary syphilis who provided these samples compared with other stages of disease (16 [31%] of 52 with primary syphilis *vs* 29 [20%] of 144 at other stages; p=0·13). Detection of *T pallidum* in the anus was more common

in men with rapid plasma reagin titres of 1/64 or higher than in those with lower titres (33 [28%] of 116 *vs* 12 [15%] of 80; p=0·037).

In the 54 men with primary syphilis, lesions occurred at the following sites: three oral, 11 perianal, two anal, one rectal, 35 penile, and two scrotal. *T pallidum* DNA was detected in the lesions of 49 (91%) of 54 primary lesions. Five men with primary syphilis had chancres that were PCR negative with positive serology. Five (9%) of 54 men with primary syphilis had *T pallidum* detected in the oral cavity. Three had a positive oral rinse sample alone, one had a positive oral lesion swab alone, and one was positive from rinse and lesion swab (figure 1). 16 (31%) of 53 men with primary syphilis had *T pallidum* detected in the anus, of whom three had a positive anal canal swab alone, seven had a positive anorectal lesion swab alone, and six were positive from both anal canal and anorectal lesion swabs (figure 1). Six (11%) of 53 men with primary syphilis who provided urine samples had *T pallidum* detected in their urine, of whom five had primary penile lesions at which *T pallidum* was detected. Three (21%) of 14 men who provided semen samples had *T pallidum* detected in their semen, all of whom had a primary penile lesion at which *T pallidum* was also detected (table 2; figure 1). All men with primary syphilis in whom we detected *T pallidum* in their urine or semen were uncircumcised. In one man with a primary anal lesion, we detected *T pallidum* both by oral rinse and in their urine sample, and in one man with a primary penile lesion, we detected *T pallidum* by oral rinse and anal canal swab and in their urine and semen samples.

78 (84%) of 93 men with secondary syphilis had a rash, of which seven (8%) were on the head, 32 (34%) were on the arms, 25 (27%) were on the palms of the hands, 62 (67%) were on the torso, 25 (27%) were on the legs, and 35 (38%) were on the soles of the feet. Additional lesions of secondary syphilis were present at the following sites: 31 oral, three anal, 19 perianal, 44 penile, and 25 scrotal. 14 (15%) of 93 men with secondary syphilis also had residual primary chancres: four oral, five penile, and five perianal.

All men with secondary syphilis had at least one swab taken from a lesion or rash, and we detected *T pallidum* at any lesion site in 48 (52%) of 93 men (table 2). In eight (9%) men we detected *T pallidum* in lesions at two sites: oral and on the genitals in five men, oral and perianal in two men, and perianal and on the palm of the hand in one man. We detected *T pallidum* from oral, anal, and genital lesions in two (2%) of 93 men. 75 (81%) men with secondary syphilis had swabs collected from rashes, and we detected *T pallidum* in only four of these samples from three men.

Notably, we detected *T pallidum* in the oral cavity of 41 (44%) of 93 men with secondary syphilis; 23 (25%) had a positive oral rinse sample alone, seven (8%) had a positive oral lesion swab alone, and 11 (12%) were positive in oral rinse samples and lesion swabs (figure 1). We detected *T pallidum* in samples taken from the anus of 27 (29%) men with secondary syphilis, of whom six (6%) had a positive anal canal swab alone, eight (9%) had a positive anorectal lesion swab alone, and 13 (14%) were positive from anal canal and anorectal lesion swabs (figure 1). Of six men with secondary syphilis in whom we detected *T pallidum* in their urine, three (50%) had secondary penile lesions from which *T pallidum* was also detected. We detected *T pallidum* in the semen of three (15%) of 20 men who provided samples, of whom one (33%) had a secondary penile lesion from which we also detected *T pallidum*. Of the eight men with secondary syphilis in whom we detected *T pallidum* in their semen or urine, or both, seven were uncircumcised.

11 (12%) of 93 men with secondary syphilis had *T pallidum* detected in two or more non-lesion samples. For one man with secondary syphilis, we detected *T pallidum* in four non-lesion samples—oral rinse, anal canal swab, urine, and semen—and from lesions in the mouth and the genital and perianal area (figure 2). In 69 (74%) men with secondary syphilis, we detected *T pallidum* from at least one site, whether from a lesion or a non-lesion sample (figure 1). We detected *T pallidum* at two or more anatomical sites in 11 (20%) of 54 men with primary syphilis, all from a lesion site and at least one other non-lesion site. No men with early latent syphilis had *T pallidum* detected at more than one site.

The oral rinse sample collection method detected an additional 28 participants with oral *T pallidum* shedding compared with the oral lesion swabbing method, of whom 23 (92%) had secondary syphilis, three (11%) had primary syphilis, and two (7%) had early latent syphilis. The anal canal swab detected an additional 11 cases of

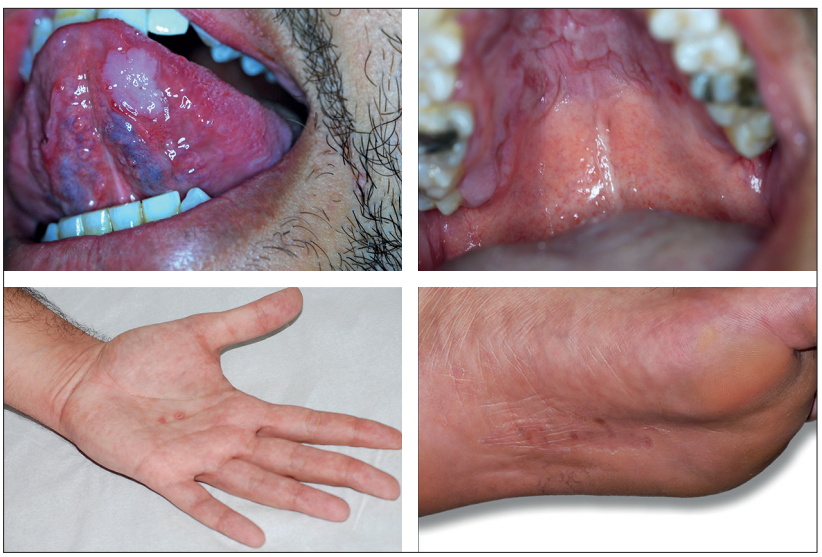

Figure 2: **Illustrative case of *Treponema pallidum* detection from multiple sites (lesion and non-lesion) in a participant with secondary syphilis**
A HIV-negative man presented with multiple oral plaques, penile and scrotal plaques, perianal plaques and condylomata lata, and a macular rash on torso, palms of the hands, and soles of the feet.

anal *T pallidum* shedding compared with the anorectal lesion swab, of whom six (55%) had secondary syphilis, three (27%) had primary syphilis, and two 918%) had early latent syphilis.

We detected *T pallidum* in non-lesion samples in four of 53 men with early latent syphilis: two with the oral rinse sample and two with the anal canal swab (figure 1).

There was 100% concordance between the *polA* and 47 kDa PCR assays for all oral rinse (n=200), urine (n=198), and semen samples (n=50). There was more than 99% concordance (195 of 196 samples) between both assays for the anal canal swab. Ct values for the 47 kDa and *polA* assays compared according to sample are in appendix 1 (p 3). We found no significant difference in *T pallidum* lesion PCR Ct values between primary and secondary syphilis lesions (p=0·069).

Discussion

We found that *T pallidum* is shed not only from the mucocutaneous lesions of MSM with primary and secondary syphilis, but also from the mouth and anus, often in the absence of overt lesions. This finding suggests asymptomatic shedding of *T pallidum* from the mouth and anus might contribute to sustaining sexual transmission of syphilis between MSM. Our findings also suggest that the secondary stage of syphilis might be the most infectious stage. We detected *T pallidum* at one or more anatomical site in 74% of men with secondary syphilis, either from a secondary lesion or from a site where no lesion was observed. In men with primary syphilis, we detected *T pallidum* at one or more anatomical site in 91% of men, and in men with early latent syphilis we detected *T pallidum* at one anatomical site in only 8% of men. In some men with secondary syphilis we detected *T pallidum* at two or even three separate sites, potentially increasing the number of infectious locations in an individual. Importantly, oral detection of *T pallidum* was highest in individuals with secondary syphilis compared with those at other stages of disease, suggesting oral transmission might be important during the secondary stage of the disease. By contrast, *T pallidum* was rarely detected from the generalised rash of secondary syphilis.

We hypothesise that *T pallidum* is shed from the oral mucosa during secondary syphilis after systemic dissemination. Although some men had oral lesions from which *T pallidum* was identified, the location of shedding from those without overt oral lesions is unknown. Detection of *T pallidum* in oral samples was greater using oral rinse than with oral mucosal swabbing, suggesting *T pallidum* might be shed from occult oral lesions or without visible mucosal disruption. In one study of predominantly HIV-positive MSM, 64·5% of men with secondary syphilis had *T pallidum* detected by oral swab.[5] Our findings indicate that oral shedding of *T pallidum* is also common among HIV-negative individuals with syphilis.

We detected *T pallidum* in anal samples in 45 (23%) of 196 men in our study, of whom ten (22%) did not have an overt anal lesion. Detection of anal *T pallidum* without lesions has been reported elsewhere.[6] This finding could indicate an occult anorectal primary lesion or subclinical shedding.[10–12] Men in our study did not have routine proctoscopy. Detection of *T pallidum* from urine and semen samples was less common and was mostly accompanied by genital lesions that were shedding *T pallidum*. Whether *T pallidum* in these samples could reflect cross-contamination from genital lesions during self-collection is unclear. *T pallidum* has been detected in urine and semen elsewhere.[13–16]

In secondary syphilis, *T pallidum* has been found in histological sections of lesions and skin scrapings from rashes.[17–20] To our knowledge, our study is the first in which the rashes of individuals with secondary syphilis have been systematically swabbed without previous abrasion to detect *T pallidum* by PCR. We used this method of sample collection because we aimed to determine the infectious potential of secondary rashes from skin-to-skin contact during sex, which would not involve skin abrasion. Because *T pallidum* was rarely detected in rashes, we postulate that sexual transmission is more likely from the mouth or anus than from the rash of individuals with secondary syphilis. A previous study of MSM suggested transmission is more likely if one man has secondary syphilis,[21] supporting our hypothesis that secondary syphilis is the most infectious stage of infection.

A strength of this study is that detection of *T pallidum* DNA at non-lesion sites was confirmed using two different gene targets. Our study also has several limitations. All participants in this study were MSM, hence further studies in women and heterosexual men and other groups are warranted to determine whether similar patterns of *T pallidum* shedding occur in other populations. Semen samples were collected after syphilis treatment, which might have affected the proportion who were positive for syphilis detected via these samples. Furthermore, to what extent *T pallidum* DNA detected by PCR is derived from viable or infectious bacteria is unknown, although if *T pallidum* DNA in the oral cavity was confirmed to be viable, it would suggest that focusing testing on earlier detection of syphilis might prevent development of the secondary stage and therefore reduce infectiousness via oral shedding. Strategies for earlier detection of syphilis to improve control should include increased detection of primary syphilis,[22] and increased serological screening of high risk individuals with higher coverage and increased frequency. Access to *T pallidum* PCR testing should be provided to health-care providers, including primary care physicians, because serology can be negative in individuals with primary syphilis,[16,20,23] and primary syphilis might be misdiagnosed as genital herpes.[23] Studies show increased serological screening of MSM can increase detection of early asymptomatic syphilis, with population-level data suggesting improved screening can prevent progression to secondary syphilis.[24–27] Although oral sex has been

implicated in transmission of syphilis previously,[28] little biological data have supported this theory.

In summary, our study suggests that occult shedding of *T pallidum* from oral and anal sites occurs in MSM with syphilis, most frequently in those with secondary syphilis. To determine the relevance of this finding to the transmission and control of syphilis, future research should examine the viability of bacteria shed from oral and anal sites, as well as the effect of treatment on the duration of oral and anal shedding of *T pallidum* and the extent to which different sexual behaviours, such as kissing, oro-genital sex, penile-anal sex, and oro-anal sex, contribute to *T pallidum* transmission.

Contributors

MYC, JMT, and DEL conceived and designed the study in consultation with the other authors. Study visits were done by RW or JMT. FA and DL did the laboratory testing. JMT analysed the data. JMT and MYC drafted the manuscript. EPFC and LZ provided advice on statistical analysis. ID assisted with staging of infection, DAW oversaw the laboratory work, CKF reviewed the statistical output, and SRG contributed to the interpretation of microbiological data. All authors except DEL, who died in 2018, critically reviewed the manuscript for important intellectual content and approved the final version. JMT and RW had access to and verified the underlying study data. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

All de-identified clinical and laboratory details are provided in appendix 1 (pp 4–19) for each participant, and further individual participant-level data will not be made available. Photographs of individual participants are available in appendix 2. The study protocol and participant consent form will be made available to researchers with a methodologically sound research proposal. The protocol and consent form will be available for 3 years from publication of this Article. All requests for data access should be made to jtowns@mshc.org.au or mchen@mshc.org.au.

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