Anal and oral detection of *Treponema pallidum* in men who have sex with men with early syphilis infection

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ABSTRACT

Objectives We aimed to characterise patterns of anal and oral detection of *Treponema pallidum* among men who have sex with men (MSM) with early syphilis.

Methods 200 MSM with serologically confirmed primary, secondary and early latent syphilis were tested with *T. pallidum polA* PCR using an anal canal swab, oral rinse, plus swabs from any anal and oral lesions in a prospective, cross-sectional study. Anal and oral *T. pallidum* cycle threshold values were compared between subsets of men and according to rapid plasma reagin (RPR) titre.

Results Of 200 men with early syphilis, 45 and 48 had anal and oral *T. pallidum* detected, respectively. Cycle threshold values were lower with anal compared with oral *T. pallidum* whether lesions were present or not. Among 27 and 42 men with anal and oral *T. pallidum* detected, respectively, and no anal or oral primary lesion, frequency of detection increased with increasing RPR titre, with 95% (25/27) and 98% (41/42) of shedding from respective sites occurring with RPR titres ≥1:16. 6.5% (13/200) of men with syphilis had concurrent detection of *T. pallidum* from both anal and oral sites: 9/13 with secondary syphilis, 7/9 of whom had anal lesions with a median duration of 30 days (range 7–180 days).

Conclusions These data suggest *T. pallidum* load at the anus is higher than at the oral cavity and that a subset of men with secondary syphilis and prolonged anal lesions may be relatively infectious. Earlier detection and treatment of syphilis, when RPR titres are lower than 1:16, could potentially reduce infectiousness from anal and oral sites.

KEY MESSAGES

⇒ Anal and oral Treponema pallidum detection in men who have sex with men with syphilis increases with increasing rapid plasma reagin titre.
⇒ Some men have concurrent anal and oral T. pallidum shedding.
⇒ T. pallidum PCR cycle threshold values are lower at the anal site compared with the oral site, indicating higher bacterial load.
⇒ T. pallidum from the anus is likely to be important in the sexual transmission of syphilis.

BACKGROUND

Over the last decade, syphilis infections have increased in many countries, leading to serious complications including neurosyphilis, ocular syphilis and congenital syphilis. In many countries men who have sex with men (MSM) have been disproportionately affected by syphilis.[1 2] In a previous study of MSM with early syphilis, we found *T. pallidum* was detected at anal and oral sites in 29% and 44%, respectively, of men with secondary syphilis, with 22% and 50%, respectively, having no visible lesions at that site, indicating that anal and oral shedding of *T. pallidum* may contribute to sustaining syphilis transmission among MSM.[3] Furthermore, 26% of men with secondary syphilis had detection of *T. pallidum* at two or more anatomical sites. These findings suggest the secondary stage of syphilis may be the most infectious. Several other recent studies have also found oral detection of *T. pallidum* to be common during the secondary stage of syphilis; however, none of these studies has also tested all men for concurrent anal *T. pallidum*.[4–6]

In this study, we performed a secondary data analysis using data from our previous work[3] and aimed to further characterise patterns of anal and oral detection of *T. pallidum* among MSM with early syphilis. Our specific objectives were the following: (1) to compare the cycle thresholds (C_T) of *T. pallidum* between anal and oral sites as a proxy for bacterial load; (2) to compare the frequency of shedding of *T. pallidum* from anal and oral sites following systemic dissemination according to rapid plasma reagin (RPR) titre and (3) to examine the clinical characteristics of men with concurrent anal and oral detection of *T. pallidum*. Our hypotheses were that: (1) the C_T of *T. pallidum* differs between anal and oral sites because of differing load and (2) some men have *T. pallidum* detection from multiple sites with prolonged syphilis lesions.

METHODS

We conducted new analyses of data from a previous study, the methods of which are reported in detail elsewhere.[3] Briefly, 200 MSM with serologically confirmed primary, secondary or early latent syphilis, and no history of antibiotic use in the previous 4 weeks, were tested for *T. pallidum* from multiple anatomical sites using clinician-collected samples and standardised collection methods. Syphilis staging was

determined by clinical signs, serology and lesion swab *T. pallidum* PCR results. Primary and secondary syphilis cases were consistent with both Australian and US case classifications, and early latent syphilis staged according to the Australian case classification, that is being of less than 2 year's duration.[7 8] As non-lesion *T. pallidum* PCR results were a non-standard test, they were not used to determine the stage. An oral rinse sample was collected using 10 mL of distilled water gargled and swilled for 15 s. In addition, a swab sample was collected from any oral lesions present. For the purposes of this study, the term 'anal' refers to all perianal, anal canal and rectal sites. A blind anal canal swab was inserted 2–3 cm into the anal canal and rotated. In addition, a swab was collected from any visible anal lesions present. *T. pallidum* detection was defined as a positive PCR result from any sample type—whether lesions were present or not, at anal or oral sites. Swabs were also collected from any genital lesions present. A comprehensive full body physical examination was performed, and all lesions and rashes were recorded on an examination proforma using standardised dermatological descriptors. Proctoscopy was only performed in two instances where there were symptoms indicating intrarectal pathology. A targeted medical history was taken, including HIV status, use of HIV pre-exposure prophylaxis (PrEP), previous syphilis infection and location and duration of any syphilis lesions present. Previous syphilis infections were confirmed from medical records.

All men had serological testing for syphilis using: *T. pallidum* particle agglutination assay (Fujirebio, Tokyo, Japan), RPR (Becton Dickinson, New Jersey, USA) and either a chemiluminescent immunoassay (DiaSorin, Saluggia, Italy) or a recombinant total antibody ELISA immunoassay (EIA) (Trepanostika EIA; BioMerieux, Marcy-l'Étoile, France) performed by the Victorian Infectious Diseases Reference Laboratory. All lesion and non-lesion (anal canal swab and oral rinse) samples were tested with a TaqMan real-time *T. pallidum polA* PCR assay.[9]

To compare *T. pallidum* C_T values at anal and oral sites, we compared median C_T in three subsets of men: (1) all *T. pallidum* detected, whether anal or oral lesions were present or absent; (2) *T. pallidum* from anal and oral lesions only and (3) *T. pallidum* excluding men where local primary anal or local primary oral lesions were present. We undertook the last analysis as we aimed to identify *T. pallidum* that had disseminated from a remote anatomical site, which we henceforth refer to as *T. pallidum* shedding.

Furthermore, we identified men who had both anal and oral detection of *T. pallidum* concurrently and recorded the site, nature and reported duration of any syphilis lesions.

Statistical analyses

The sample size of 200 men was determined on the basis of 95% CIs around the 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.[6] Data were analysed using SPSS (Armonk, New York, USA; IBM, V.27). Categorical variables (eg, oral and anal detection) were compared using Fisher's exact test. The Mann-Whitney U test was used to compare continuous variables (eg, *T. pallidum* PCR C_T values) between sites.

RESULTS

Comparison of anal and oral *T. pallidum* C_T values

Among 200 men with early syphilis, there were 45 with anal *T. pallidum* detected including 16/45 with primary syphilis, 27/45 with secondary syphilis and 2/45 with early latent syphilis. 10/45 (22%) were HIV positive and 8/45 (18%) were repeat syphilis infections. Among 200 men with early syphilis, there were 48 with oral *T. pallidum* detected including 5/48 with primary syphilis, 41/48 with secondary syphilis and 2/48 with early latent syphilis. 9/48 (19%) were HIV positive and 11/48 (23%) were repeat syphilis infections.

C_T values for anal samples were significantly lower than that for oral samples, indicating higher *T. pallidum* loads at the anus compared with the oral cavity. This applied when comparing anal and oral samples using three ways to categorise detection: (1) when anal and oral primaries were included (median C_T 32 and 35, respectively, IQRs 30–34 and 33–36.75, respectively, p=0.0007); (2) when only anal and oral lesion samples were included (median C_T 32 and 35, respectively, IQR 30–35 and 33–37.5, respectively, p=0.022) and (3) when anal and oral primaries were excluded (median C_T 32 and 35, respectively, IQR 30–35 and 33–36.25, respectively, p=0.017) (figure 1). C_T values from anal lesion samples were significantly lower than that from genital lesion samples, indicating higher *T. pallidum* loads from anal compared with genital lesions (C_T 32 and 35, respectively, p=0.02). There were no significant differences in C_T values comparing anal lesion swabs with anal canal swabs (p=0.504); oral lesion swabs with oral rinse samples (p=0.956) or oral lesion swabs with genital lesion swabs (p=0.513).

Anal and oral *T. pallidum* shedding by RPR titre excluding local primary lesions

There were 27 men with anal *T. pallidum* shedding, as detected by PCR, after excluding 18 men with local primary anal lesions. The 27 men included 3 with primary syphilis (with primary lesions that were not anal), 22 with secondary syphilis and 2 with early latent syphilis. Seven of the 27 (26%) were HIV positive and 5 (19%) were repeat syphilis infections. RPR titres ranged from 1:2 to ≥1:512, with a median titre of 1:128. The frequency of anal *T. pallidum* shedding by RPR titre is shown in figure 2 and online supplemental table 1. Ninety five per cent (25/27) of men with anal *T. pallidum* shedding had an RPR titre of 1:16 or higher.

There were 42 men with oral *T. pallidum* shedding, as detected by PCR, after excluding 6 men with local oral primary lesions, with 7 having shedding at both anal and oral sites. The 42 men included 3 with primary syphilis (with primary lesions that were not oral), 37 with secondary syphilis and 2 with early latent syphilis. Nine of the 42 (21%) were HIV positive and 9 (21%) were repeat syphilis infections. RPR titres ranged from 1:8 to >1:512, with a median titre of 1:128. The frequency of oral *T. pallidum* shedding by RPR titre is shown in figure 2 and online supplemental table 2. Ninety eight per cent (41/42) of men with oral *T. pallidum* shedding had an RPR titre of 1:16 or higher.

Concurrent oral and anal *T. pallidum* detection

Thirteen (6.5%) of the 200 men with early syphilis had concurrent detection of *T. pallidum* from both oral and anal sites: 4 with primary syphilis and 9 with secondary syphilis. Four men with primary syphilis presented with perianal, rectal, tonsillar and penile primary lesions. Of the 13 men, 2 were HIV positive and 11 HIV negative, including 3 taking HIV PrEP. Two had a repeat syphilis infection. The RPR titre ranged from 1:8 to 1:256, with a median titre of 1:128. The clinical characteristics of nine men with secondary syphilis and concurrent anal and oral *T. pallidum* detection are shown in table 1. Of these nine men, seven had anal lesions, including four with anal condylomata lata. The median duration of anal lesions reported was 30 days, ranging from 7 to 180 days. In five men, the duration of all types of anal lesions was longer than the duration of lesions at other anatomical sites, see table 1. Seven men had *T. pallidum*

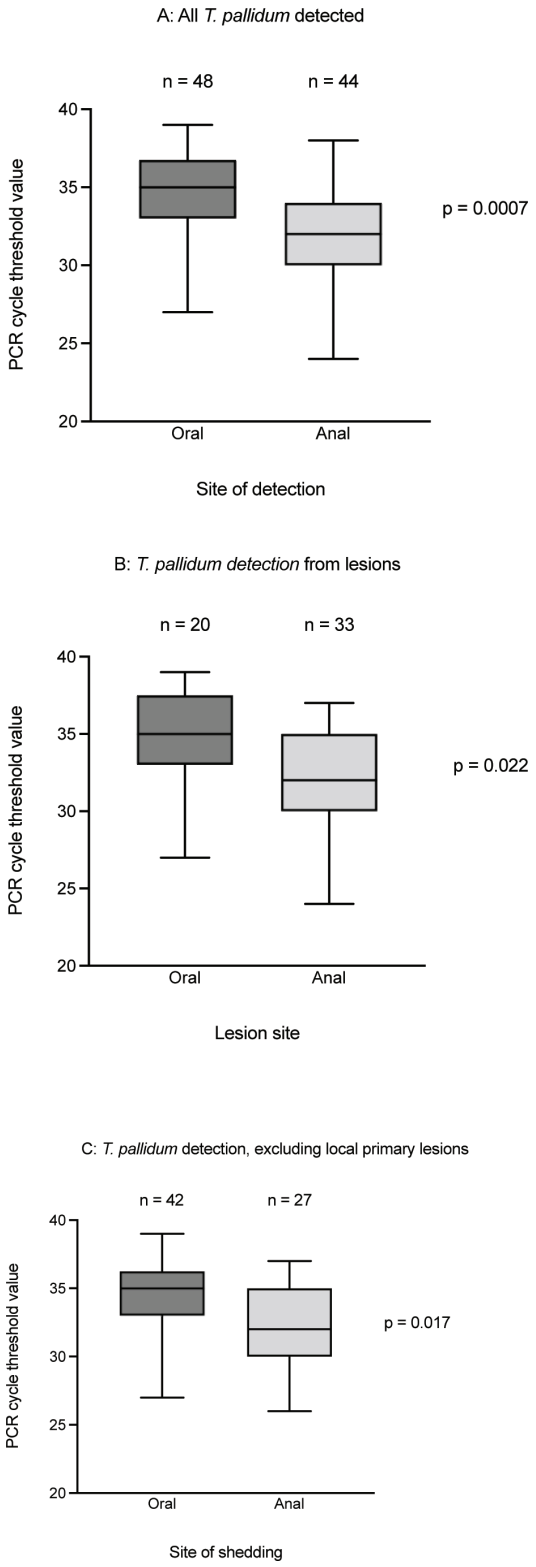

Figure 1 Comparison of *Treponema pallidum polA* PCR cycle threshold values between: (A) all oral and anal *T. pallidum* detected (B); *T. pallidum* detected from oral and anal lesions only and (C) *T. pallidum* detection from oral and anal sites excluding local primary lesions. (A) All *T. pallidum* detected, whether primary lesions at anal or oral sites were present or absent; (B) *T. pallidum* detected only where anal or oral lesions were present; (C) *T. pallidum* detected if there was no primary lesion at anal or oral site. n is the number in category. Box and whisker plots show maximum, minimum, median, 25th and 75th quartile points.

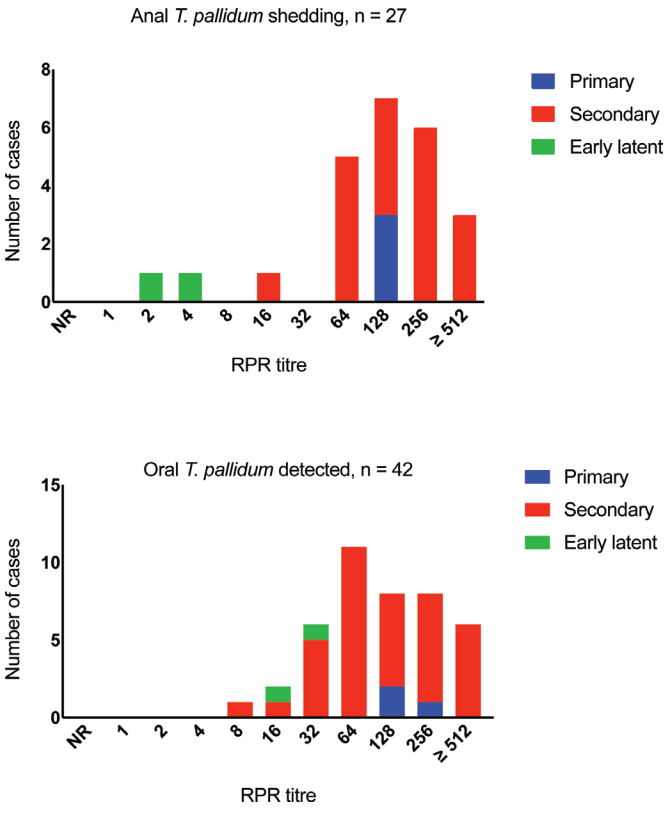

Figure 2 The frequency of anal and oral *T. pallidum* shedding by RPR titre*. *Men with local anal primary and local oral primary lesions were excluded to identify *T. pallidum* that had disseminated from a remote anatomical site. NR, non-reactive; RPR, rapid plasma reagin.

detected at two sites, one at four sites (oral, genital, anal and foot) and one in five different samples (oral, genital, anal, urine and semen).

CONCLUSIONS

In this cross-sectional study of MSM with syphilis, we show that shedding of *T. pallidum* from anal and oral sites increased with rising RPR titre, with most shedding occurring in men with secondary syphilis and almost all occurring at RPR titres ≥1:16. In this analysis we excluded men with local primary anal lesions and local primary oral lesions with the aim of capturing the pattern of *T. pallidum* shedding from these sites following systemic dissemination from a remote site of infection. In a recent study of individuals with syphilis by Wang *et al*, detection of *T. pallidum* from saliva and blood using droplet digital PCR both peaked during secondary syphilis, providing evidence that *T. pallidum* is shed from the oral mucosa following systemic dissemination.[4] We postulate that *T. pallidum* may also be shed from the anal region following systemic dissemination; however, this needs to be verified in studies using assays that can detect blood and anal *T. pallidum* with high sensitivity.

We found that median *T. pallidum* C_T values were significantly lower at the anal site compared with median *T. pallidum* C_T values from the oral cavity, and this applied whether or not anal or oral lesions were present. Furthermore, median C_T values from anal lesions were lower than the median C_T values in genital lesions. The lower C_T values of *T. pallidum* from the anal site are an indirect proxy measurement indicating higher bacterial loads at the anus. These findings suggest transmission of *T. pallidum*

Table 1 Clinical and laboratory characteristics of nine men with secondary syphilis and concurrent oral and anal *Treponema pallidum* detection

Case no.	HIV status	RPR	Reinfection	Lesion site	Symptom duration at site (days)
1	Negative	64	No	Palate ulcer	2
				Perianal condylomata lata	120
				Generalised rash torso, palms	14
2	Negative	64	No	Oral plaque	7
				Perianal condylomata lata	7
				Scrotal plaque	3
				Macular rash palms, soles	14
3	Positive	256	Yes	Tongue ulcers	30
				Perianal condylomata lata	30
4	Negative	128	No	Tonsillar ulcers	U
				Penile glans ulcers	7
				Sole macule	U
5	Positive	128	No	Penile glans plaques	14
6	Negative	8	No	Inner lip ulcer	U
				Perianal chancre	30
				Generalised rash torso	U
7	Negative	256	Yes	Tonsillar chancre	3
				Penile plaques	14
				Perianal ulcers	90
				Generalised rash torso	14
8	Negative	32	No	Penile shaft macules	U
				Perianal chancre, condylomata lata	21
				Generalised rash torso	14
9	Negative	256	Yes	Inner lip and tongue ulcers	180
				Penile shaft ulcers	7
				Perianal ulcers	180
				Desquamation soles	180

RPR, rapid plasma reagin; U, unknown.

from the anus is likely to be important in the sexual transmission of syphilis.

We identified a subset of men who had concurrent detection of *T. pallidum* from both anal and oral sites. Most of these men had secondary syphilis, several of whom had detection from multiple anatomical sites together with anal lesions such as condylomata lata that had been present for up to several months. We postulate that such men are potentially highly contagious and may contribute disproportionately to syphilis transmission. However, this hypothesis needs to be examined and verified through further studies, such as studies of transmission networks for syphilis.

There are a number of limitations to this study. First, PCR C_T values for *T. pallidum* are semiquantitative, providing an indirect proxy for bacterial load. Also, bacterial load may vary according to the adequacy of sampling of lesions or non-lesion sites. The method of collection for oral rinse, anal canal swab and lesions was standardised to minimise variations in sampling technique, but it is possible that site-specific collection methods may have contributed to differences in detected organism loads. Second, while we have shown *T. pallidum* shedding from anal and oral sites increases with RPR titre, we should point out this is not a function of time. The period of time between infection and shedding of *T. pallidum* in the study is not known. Our data are derived from a cross-sectional study: the natural history of shedding over time would need to be from a longitudinal cohort of individuals with untreated syphilis, which may not be ethically possible to undertake. Third, in our analysis of *T. pallidum* shedding by RPR titre we aimed to capture *T. pallidum* that had disseminated from a remote site of inoculation by excluding local anal and local oral primary lesions. Proctoscopy was not routinely performed, and it is also possible some local anal or oral primary lesions had resolved or were occult, resulting in inclusion of *T. pallidum* detected from local infection. Fourth, it is possible that some of the multisite detection has arisen from inoculation at multiple sites at the time of sexual exposure, but it is unknown how commonly this occurs. Fifth, the sensitivity of *T. pallidum* detection may vary by assay used. Using nested PCR and digital droplet PCR, *T. pallidum* was detected in the saliva of 87.5% of individuals with secondary syphilis with the highest loads of *T. pallidum* during secondary syphilis.[4] Sixth, detection by PCR does not necessarily relate to viability or transmissibility of *T. pallidum*, and viability studies should be undertaken to confirm this.

In summary, our study suggests *T. pallidum* shedding from the anal region is likely to be an important in the sexual transmission of syphilis between men. Moreover, we postulate that some men who have *T. pallidum* detectable from multiple locations including anal and oral sites and who have prolonged anal lesions may be infectious for a relatively long period of time, potentially contributing disproportionately to transmission. As most asymptomatic detection of *T. pallidum* from anal and oral sites appears to occur following an RPR titre of 16 or higher, earlier detection and treatment of syphilis, when titres are lower, could potentially reduce infectiousness from anal and oral sites. Further studies that provide evidence for this strategy are warranted.

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Contributors JMT and MYC conceived the idea for this study. Study visits were done by RW or JMT. JMT analysed the data. JMT and MYC drafted the manuscript. EPFC and LZ provided advice on statistical analysis. DW oversaw the laboratory work, FA assisted with laboratory testing and methodology, CKF reviewed the statistical output, and SG contributed to the interpretation of microbiological data. All authors 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. JMT is responsible for the overall content as the guarantor.

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Competing interests MYC and DW have received donated materials from SpeeDx and Hologic.

Patient consent for publication Not required.

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