**Shigella** is common in symptomatic and asymptomatic men who have sex with men visiting a sexual health clinic in Amsterdam

Joyce F. Braam, Sylvia M. Bruisten, Mariska Hoogeland, Henry J.C. de Vries, Maarten F. Schim van der Loeff, Alje P. van Dam

**ABSTRACT**

**Introduction** Shigellosis is a reportable infectious disease. It can present as a severe bloody diarrhoea but is often asymptomatic. **Shigella** can be sexually transmissible. We performed a study among symptomatic and asymptomatic men who have sex with men (MSM) to assess the prevalence of **Shigella**, **Salmonella** and **Campylobacter**.

**Methods** From March to June 2020, MSM attending the Amsterdam centre for sexual health were consecutively included. Predefined minimal numbers of inclusion of 150, 100 and 50 were determined, respectively, for MSM who reported no diarrhoea, diarrhoea during last month or diarrhoea on the day of visit to clinic. Anal samples were tested for the presence of **Shigella**, **Salmonella** and **Campylobacter**. During the same period, the frequency of these bacteria was assessed in routinely tested samples requested by general physicians or nursing home physicians. Characteristics of included MSM were compared between the men with different diarrhoea anamnesis, and the prevalence of shigellosis was estimated in each group.

**Results** We included 212 MSM without diarrhoea, 109 MSM who recently had diarrhoea and 68 MSM who reported diarrhoea on the day of clinic visit. Thirteen (3.3%, 95% CI 1.7% to 5.6%) MSM were infected with **Shigella**, none with **Salmonella** and 7 (1.8%, 95% CI 0.7% to 3.7%) with **Campylobacter**. **Shigella** prevalence was 2.8% (95% CI 1.0% to 6.1%) in asymptomatic men, 3.7% (95% CI 1.0% to 9.1%) in men who recently had diarrhoea and 4.4% (95% CI 0.9% to 12.4%) in men with current diarrhoea (p=0.799). **Shigella** was more frequently found in MSM who had used pre-exposure prophylaxis (PrEP) in the preceding 3 months (10/151), compared with those not having used PrEP (2/146) or being HIV positive (1/75) (p=0.038). **Shigella** was significantly more often detected among MSM compared with routinely obtained faecal samples being 11/770 (1.4%) (p=0.031).

**Conclusion** **Shigella** infections are relatively common in both symptomatic and asymptomatic MSM. Future studies should focus on the risk of onward transmission via asymptomatic persons.

**INTRODUCTION**

**Shigella** is an important cause of bacterial diarrhoea. Shigellosis, also known as bacillary dysentery, is a reportable infectious disease in the Netherlands and can present as a severe bloody diarrhoea. Not
all infections result in clinical symptoms and not all persons with clinical symptoms are diagnosed. In Western countries, imported and domestically acquired shigellosis causes a substantial health-related burden such as disease control measures, use of healthcare facilities and a high number of disability adjusted life years.

In many countries, including the Netherlands, isolation of *Shigella* is required for notification of shigellosis. The sensitivity of culturing is limited, but antimicrobial susceptibility testing is important, as *Shigella* species are developing resistance against several antibiotic groups. *Shigella* can be detected by molecular methods targeting the *ipaH* gene, but a distinction with entero-invasive *Escherichia coli* (EIEC) cannot be made with this technique. However, both *Shigella* and EIEC can cause serious disease, and public health consequences of EIEC and *Shigella* infections are similar.

About 22% of reported shigellosis cases in the Netherlands occur in men who have sex with men (MSM). In a Danish study, MSM had a matched OR (mOR) of 105 of *Shigella* infections compared with controls. In a US study on gastroenteritis in MSM, 30% had shigellosis. Several outbreaks of multidrug-resistant *Shigella* strains, such as with *S. flexneri* 3a in England, have been reported among MSM. Sexual behaviour such as oro-anal contact, condomless anal intercourse, multiple sexual partners, chemsex and encounters arranged through mobile dating applications are associated with shigellosis outbreaks among MSM.

In the Netherlands in 2009–2016, 360–752 shigellosis cases yearly were confirmed and notified. The real number of cases is expected to be much higher. Haagsma et al estimated substantial underreporting, especially if diarrhoea is not bloody and caused by the limited sensitivity of culture; for every notified *Shigella* case, approximately 53 cases may go undiagnosed.

Duration of persistence of *Shigella* after diarrhoea is generally unknown. Persistence of *Shigella* after 30 days in children in a low-income country was as high as 79%, but this could be related to persistent contamination of food or water.

In adults in high-income countries, no systematic studies regarding duration of *Shigella* carriage or *Shigella* PCR positivity have been done, although *Shigella* long-term carrier state has been described. So possibly in a population with a high frequency of Shigellosis, such as MSM, *Shigella* may be regularly detected in stool during but also shortly after an episode of diarrhoea.

Asymptomatic infections with *Shigella* seem to be uncommon in the general population. In a previous Dutch study, *Shigella* was detected in 0.9% of patients with diarrhoea (n=1515) but not at all in healthy controls (n=1195). However, this study did not focus on key populations like MSM. Little is known whether and how often asymptomatic *Shigella* infections occur in MSM.

*Salmonella* and *Campylobacter* have also been reported among MSM and to be sexually transmissible. The most common detected bacteria in stool samples from MSM with gastroenteritis in the USA were diarrheagenic *Escherichia coli* (33%), *Shigella* (30%) and *Campylobacter* (17%). In a Danish study, MSM had a mOR of 16 for *Campylobacter* infection compared with controls, but no significantly increased mOR was found for *Salmonella* infection. Since *Salmonella* and *Campylobacter* are not notifiable pathogens in The Netherlands, it is unknown what the prevalence or incidence of these infections are among MSM.

Targeted research into pathogens in MSM with or without diarrhoea has not been conducted in Amsterdam before. Therefore, we performed a study among MSM visiting the STI clinic in Amsterdam to assess the prevalence of *Shigella*, *Salmonella* and *Campylobacter*.

**METHODS**

**Study design and inclusion criteria**

This prospective study included MSM ≥16 years old attending the STI clinic in Amsterdam in March–June 2020. We aimed to include samples of 150 asymptomatic men, 100 men with diarrhoea in the previous month and 50 with diarrhoea on the day of clinic visit (see further). Men from the different diarrhoea categories were consecutively included, until the target number for that category was reached. Diarrhoea was defined as thin or watery stools, or bowel passages more than two times daily. Age, ethnicity, sexual behaviour, STI diagnoses, HIV status, pre-exposure prophylaxis (PrEP) use and the presence of clinical symptoms were extracted from electronic patient files.

**Study sample size**

As a reference, we used the prevalence of 0/1195 (0%, 95% CI 0% to 0.3%) *Shigella* infections in healthy controls in the general population in another Dutch study. We assumed a prevalence of 2% *Shigella* among asymptomatic MSM. As a reference for the prevalence of shigellosis in MSM with diarrhoea, we used 30% as found in a study conducted in the USA and divided this by two since we expect a lower prevalence in the Netherlands, resulting in an expected prevalence of 1% among MSM with diarrhoea. In order to determine a statistically significant difference between the prevalence of *Shigella* in asymptomatic MSM and MSM with current diarrhoea, we would have to include 150 and 50 persons, respectively. We estimated that the *Shigella* prevalence would decline by 50% in the first month after diarrhoea and therefore aimed to include 100 MSM with recent diarrhoea.

**Detection of bacterial pathogens**

Anal swab samples were routinely taken to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* with the Aptima Combo 2 (AC2) transcription-mediated amplification assay (Hologic Inc, San Diego, California, USA) according to the manufacturer’s instructions. Use of routinely taken anal swabs to detect *C. trachomatis* and next to detect gastrointestinal infections has been used before. DNA was extracted with the Magna Pure 24 System (Roche, Rotkreuz, Switzerland) and tested for the presence of *Shigella*/EIEC by qPCR on the *ipaH* gene, *Salmonella* on the *ttrC* gene and *Campylobacter* on the *mapA* and *ceuE* genes. Primers and probes are listed in table 1. Amplification was performed on aRotorGene (Qiagen, Hilden, Germany) with the following program for *Shigella*, *Salmonella* and *Campylobacter*: 2 min at 50°C, 2 min at 95°C followed by 45 cycles of 95°C for 15 s and 60°C for 45 s. Since clinical symptoms and public health consequences of *Shigella* and EIEC are similar, positive *ipaH* qPCR results are reported as *Shigella* infections throughout the remainder of the manuscript. A sample was considered positive for *Campylobacter* if one or both of the genes were positive with qPCR. *Shigella*, *Salmonella* and *Campylobacter* testing were done for study purposes only, and results were neither disclosed to the men nor to the healthcare professionals. During the study period, the frequency of *Shigella*, *Salmonella* and *Campylobacter* was assessed by qPCR in routinely tested samples requested by general physicians or nursing home physicians. For them, the main and almost only reason to send a faecal sample for testing for *Shigella*, *Salmonella* and *Campylobacter* is diarrhoea. No specific clinical information about symptoms was available from these routinely tested samples. Only shigellosis is a notifiable condition.
disease in the Netherlands. We collected data regarding the number of notified cases and distributions among different categories in the Amsterdam region.

Statistical analysis
Characteristics of included MSM clients were compared between the men with different diarrhoea anamnesis, and the prevalence of shigellosis was estimated in each group. Also, prevalence was estimated and compared between groups of demographic and sexual behavioural variables, with 95% CIs. Differences were assessed with the $\chi^2$ test or Fisher’s exact test, except for age, which was compared using the Kruskal-Wall test. Univariable logistic regression analysis was performed to identify possible risk factors for shigellosis. The prevalence of shigellosis was compared between MSM and routinely tested samples requested by general physicians or nursing home physicians in the study period, using the $\chi^2$ test. Level of significance was set at $p<0.05$. Data were analysed using Stata Intercooled 15 (StataCorp LLC, Texas, USA).

Ethics statement
Men of the STI outpatient clinic Amsterdam were informed of the ‘opt-out’ system regarding research on remnants of patient material. All data were pseudonymised before analysis.

RESULTS
Study population characteristics
During the study period 2927 MSM clients visited the clinic, of whom 2307 (78.8%) reported no diarrhoea, 512 (17.5%) reported diarrhoea in the month before the visit and 110 (3.7%) reported diarrhoea on the day of the visit. We included samples from 212 MSM without diarrhoea, who were included 3–9 March 2020, 109 MSM who had diarrhoea in the preceding month included 3–16 March 2020 and 68 MSM who reported diarrhoea on the day of clinic visit, included 3 March–23 June 2020 (table 2). Of note, nationwide lockdown measures to inhibit SARS-CoV-2 transmission started on 16 March. Thus, most of the samples from MSM with diarrhoea were collected during the lockdown period. Median age was 32 years (IQR 26–44), 41.4% (161) were MSM of Dutch origin, 38.8% (151) reported PrEP use, 19.3% (75) were HIV-positive and 7.5% (29) reported anal symptoms, other than diarrhoea.

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>Gene</th>
<th>Primer/probe</th>
<th>Sequence 5’−3’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shigella/EIEC</td>
<td>ipaH</td>
<td>Forward</td>
<td>CCT TTT CCG CGT TCC TTG A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>CCG AAT CCG GAG GTA TTG C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Probe</td>
<td>ROX - CGCCTTCCCGGATACCTCTGCA - BHQ2</td>
</tr>
<tr>
<td>Salmonella</td>
<td>traC</td>
<td>Forward</td>
<td>CTC ACC AGG AGA TTA CAA CAT GG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>AGC TCA GAC CAA AAG TGA CCA TC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Probe</td>
<td>JOE-CACCGACCCGAGACCCCACCTT-TAM</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>mapa</td>
<td>Forward</td>
<td>CGT GTG GTG TTG AGA CAA AGA TT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>CAA TAC CAG TGT CTA AAG TGA GTC TTAT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Probe</td>
<td>FAM - AAT TCC AAT AAT AAT GTG A</td>
</tr>
<tr>
<td>Campylobacter coli</td>
<td>ceuE</td>
<td>Forward</td>
<td>TCT CGG TTT GGA ATC AAT CAT G</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>CAT GTG TGC CTA CTT TTA CAT CTT CA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Probe</td>
<td>FAM - TGT TIT AGG AAT CAA TGC TGT</td>
</tr>
</tbody>
</table>

Table was created by the authors.

EIEC, enteroinvasive Escherichia coli.

Shigella infections
The total number of MSM infected by Shigella was 13/389 (3.3%; 95% CI 1.7% to 5.6%). Of MSM without diarrhoea 6/212 (2.8%; 95% CI 1.0% to 6.1%) were infected, 4/109 (3.7%; 95% CI 1.0% to 9.1%) of MSM who recently had diarrhoea and 3/68 (4.4%; 95% CI 0.9% to 12.4%) of MSM who had diarrhoea on the day of clinic visit ($p=0.799$) (table 2). During the study period, 11/770 (1.4%; 95% CI 0.7% to 2.5%) of routinely tested faecal samples sent by general physicians or nursing home physicians were positive for Shigella, significantly lower in comparison with MSM included in this study ($p=0.031$). Shigella positive samples were more frequently found in HIV negative MSM using or recently having used PrEP (10/151, 6.6%), compared with HIV negative MSM not using PrEP (2/146, 1.4%) or HIV positive MSM (1/75, 1.3%) ($p=0.038$) (table 3). No association was found between number of sex partners (0–1 vs 2 or more) in last 6 months, age, country of origin or coinfections. Only 3/13 (23.1%) of the men with Shigella had diarrhoea; one of them also had anal discharge and was diagnosed with syphilis. The other 10 men with a Shigella infection reported no symptoms. Three of 13 MSM with Shigella (23.1%) had a coinfection with C. trachomatis, one (7.7%) with N. gonorrhoeae and one (7.7%) with Treponema pallidum. Coinfections with hepatitis B, Campylobacter, Salmonella or SARS-CoV-2 were not observed. The number of notified Shigella infections among the entire population of Amsterdam region was 48 in 2020, of which 2.3 were from MSM, 15 were travel related and for 10 none of these applied.

Salmonella infections
We detected no Salmonella infections in the included MSM population. In comparison, in routinely tested faecal samples among the same study period, 3/771 (0.4%; 95% CI 0.1 to 1.1%) samples were positive for Salmonella ($p=0.355$). From the MSM included in the study, 7/389 (1.8%; 95% CI 0.7% to 3.7%) were infected by Campylobacter. Infection with Campylobacter was 0.9% (2/212) among MSM without diarrhoea, 3.7% (4/109) among MSM reported to have had recently diarrhoea, and 1.5% (1/68) among MSM with diarrhoea at clinic visit. In comparison to all MSM included in this study, 19/770 (2.5%; 95% CI 1.5% to 3.8%) of routinely tested faecal samples during the study period were positive for Campylobacter ($p=0.468$).
DISCUSSION
We found a positivity rate of 3.3% *Shigella* and 1.8% *Campylobacter* among MSM attending the STI clinic in Amsterdam in the spring of 2020. We did not detect any *Salmonella* infection during the study period. *Shigella* infections were significantly more prevalent among MSM compared with routinely tested samples requested by general physicians or nursing homes, and these routinely tested samples were most likely from symptomatic patients. No difference regarding *Campylobacter* infections was found. In this study population, none of the pathogens were significantly associated with diarrhea at clinic visit or in the last month.

No significant difference in *Shigella* prevalence was found between MSM with current or recent diarrhea compared with MSM without diarrhea. This could well be due to a lack of power because of the small sample size. However, our findings suggest that shigellosis in MSM is frequently asymptomatic. *Shigella*-positive samples were more frequently found in MSM with diarrhoea last month or currently diarrhoea, compared with MSM without diarrhoea. This could well be due to a lack of power because of the small sample size. However, our findings suggest that shigellosis in MSM is frequently symptomatic.

### Table 2

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n=389)</th>
<th>No diarrhea (n=212)</th>
<th>Diarrhea last month (n=109)</th>
<th>Currently diarrhoea (n=68)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, median (IQR)</td>
<td>32 (26–44)</td>
<td>33 (26–46)</td>
<td>33 (26–44)</td>
<td>29 (26–37.5)</td>
<td>0.088</td>
</tr>
<tr>
<td>Age in years, n (%)</td>
<td>16–24</td>
<td>67 (17.2)</td>
<td>37 (17.5)</td>
<td>17 (15.6)</td>
<td>13 (19.1)</td>
</tr>
<tr>
<td>Country of origin, n (%)</td>
<td>Netherlands</td>
<td>161 (41.4)</td>
<td>91 (42.9)</td>
<td>53 (48.6)</td>
<td>17 (25.0)</td>
</tr>
<tr>
<td>HIV and PrEP status, n (%)</td>
<td>HIV−, no PrEP use</td>
<td>146 (37.5)</td>
<td>81 (38.2)</td>
<td>40 (36.8)</td>
<td>25 (36.8)</td>
</tr>
<tr>
<td>No. of sex partners in last 6 months, n (%)</td>
<td>0 or 1</td>
<td>35 (9.0)</td>
<td>20 (9.4)</td>
<td>10 (9.2)</td>
<td>5 (7.4)</td>
</tr>
<tr>
<td>Anal symptoms, n (%)</td>
<td>No</td>
<td>360 (92.5)</td>
<td>210 (99.1)</td>
<td>102 (93.6)</td>
<td>48 (70.6)</td>
</tr>
<tr>
<td>CT, n (%)</td>
<td>Negative</td>
<td>354 (91.0)</td>
<td>211 (99.5)</td>
<td>100 (91.7)</td>
<td>60 (88.2)</td>
</tr>
<tr>
<td>NG†, n (%)</td>
<td>Negative</td>
<td>339 (87.2)</td>
<td>183 (86.3)</td>
<td>100 (91.7)</td>
<td>56 (82.4)</td>
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<td>Negative</td>
<td>354 (91.0)</td>
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<td>56 (82.4)</td>
</tr>
</tbody>
</table>

*P values were determined excluding the unknown values.
†Pathogen detected on any anatomical location.
CT, *Chlamydia trachomatis*; MSM, men having sex with men; NG, *Neisseria gonorrhoeae*; PrEP, pre-exposure prophylaxis.

Table was created by the authors.
HIV-negative MSM with recent PrEP use, compared with HIV-negative MSM not using PrEP or HIV positive MSM. This might be due to an higher number of sexual partners among PrEP users (115/151, 76.8%) had five or more sex partners in the last 6 months compared with no PrEP users (73/146, 50.0%) had five or more sex partners in the last 6 months), potentially including a higher frequency of oro-anal contacts. However, many HIV-positive MSM similarly had five or more sex partners in the last 6 months (60/75, 80.0%). We did not find a correlation between positive MSM and the number of sexual partners. However, we discriminated only between 0–1 and 2 or more sexual partners in the last 6 months (60/75, 80.0%). We did not find a correlation between positive MSM not using PrEP or HIV positive MSM. This might be expected that during the 16-week study period, it can be expected that during the 16-week study period 65 asymptomatic (95% CI 24 to 140), 19 (95% CI 5 to 47) recently symptomatic and 5 (95% CI 1 to 14) symptomatic MSM were infected with Shigella. Thus, in total, 89 men (95% CI 30 to 210) out of the 2929 MSM clients attending the STI clinic in a 16-week period might have been infected with Shigella. Considering that in 2020 in Amsterdam region 48 cases of shigellosis were notified, of which 23 were from MSM, this suggest that the actual prevalence of shigellosis is much higher than the number of notified cases. A positive culture is mandatory for the notification and cultures often remain negative, due to low load or to the presence of EIEC causing the positive PCR result.

Previously, Shigella was detected in 0.9% of patients with diarrhoea in the Netherlands but not detected in healthy controls. In contrast, we found that 2.8% of the MSM without diarrhoea were infected with Shigella, which was not significantly different compared with MSM with diarrhoea or who had diarrhoea in the previous month. Thus, asymptomatic Shigella infections do occur relatively frequently in MSM. The impact of diagnosing asymptomatic Shigella infections depends highly on the frequency that they will cause secondary, symptomatic infections. This should be further investigated.

In our study, the prevalence of Campylobacter and Salmonella infections were lower in MSM with or without diarrhoea compared with what was previously reported. In a Danish study, Campylobacter and Shigella infections were associated with being MSM, but Salmonella infection was not. Whether or not sexual transmission plays a significant role in Campylobacter and Salmonella infections is still unclear.

A limitation of this study is that because of the start of the SARS-CoV-2 epidemic in the Netherlands, our results might not be fully translatable to a time without an epidemic. Since the national lockdown on 16 March 2020, international travel was highly restricted, bars and restaurants were closed and advice
was given against social encounters. This lockdown lasted until after the end of our study period, although some restrictions were eased from 1 June 2020, onwards. Access to the STI clinic in that period was highly restricted and only persons with clinical symptoms and persons at high risk of STIs were seen at the clinic. All samples from persons without diarrhoea and the majority of persons with recent diarrhoea had already been collected before the onset of the lockdown. A large proportion of samples from patients with diarrhoea at clinic visit, however, were collected during the lockdown period, during which the epidemiology of infectious diseases was possibly changed and less Shigella may have circulated. Another limitation of this study is that we could not make a distinction between Shigella and EIEC infections. A strength of this study is that we did not only take into account diarrhoea at time of visit, but also diarrhoea in the previous month.

To conclude, Shigella is prevalent among both symptomatic and asymptomatic MSM. Whether and when MSM should be tested for Shigella in MSM to prevent the spread of this notified pathogen remains unclear. Future studies should focus on the risk of Shigella transmission of asymptomatic MSM, possibly leading to symptomatic infections in their partners.

Key messages

⇒ Men who have sex with men (MSM) have Shigella infections more often than the general population.
⇒ In the Netherlands, prevalence of Shigella among symptomatic (4.4%) and also asymptomatic (2.8%) MSM is high.
⇒ Shigella is more frequently found in MSM who used pre-exposure prophylaxis (PrEP), compared with those without a history of PrEP use and HIV-positive men.
⇒ Salmonella and Campylobacter were not found more frequently in MSM than the general population.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants, but Medical Ethics Committee of the University Amsterdam Medical Centers W20_151 # 20.167 exempted this study. Men of the STI outpatient clinic Amsterdam were informed of the ‘opt-out’ system regarding research on remnants of patient material. All data were pseudonymised before analysis.

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