

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

Joyce F. Braam ¹, Sylvia M. Bruisten ^{1,2}, Mariska Hoogeland,¹
Henry J.C. de Vries ^{1,3}, Maarten F. Schim van der Loeff ^{1,4}, Alje P. van Dam^{1,2}

¹Department of Infectious Diseases, Public Health Service of Amsterdam, Amsterdam, The Netherlands

²Department of Medical Microbiology, Amsterdam UMC Locatie AMC, Amsterdam, North Holland, The Netherlands

³Department of Dermatology, Amsterdam UMC Locatie AMC, Amsterdam, North Holland, The Netherlands

⁴Department of Internal Medicine, Amsterdam UMC Locatie AMC, Amsterdam, North Holland, The Netherlands

Correspondence to

Joyce F. Braam, Department of Infectious Diseases, Public Health Service of Amsterdam, 1018 WT Amsterdam, The Netherlands; jbraam@ggd.amsterdam.nl

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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.

Samenvatting

Introductie

Shigellose is een meldingsplichtige infectieziekte. Het kan zich presenteren als een ernstige bloederige diarree, maar is vaak asymptomatisch. *Shigella* kan

seksueel overdraagbaar zijn. We hebben een onderzoek uitgevoerd onder symptomatische en asymptomatische mannen die seks hebben met mannen (MSM) om de prevalentie van *Shigella*, *Salmonella* en *Campylobacter* te bepalen.

Methoden

Van maart tot juni 2020 werden achtereenvolgens MSM van het Amsterdamse centrum voor seksuele gezondheid opgenomen. Vooraf gedefinieerde minimale aantallen van inclusie van respectievelijk 150, 100 en 50 waren bepaald voor MSM die geen diarree, diarree in de afgelopen maand of diarree op de dag van bezoek aan de kliniek meldden. Anale monsters werden getest op de aanwezigheid van *Shigella*, *Salmonella* en *Campylobacter*. In dezelfde periode werd de frequentie van deze bacteriën bepaald in routinematig geteste monsters aangevraagd door huisartsen of verpleeghuisartsen. Kenmerken van geïncludeerde MSM werden vergeleken tussen mannen met verschillende diarree anamnese, en de prevalentie van shigellose werd in elke groep geschat.

Resultaten

We includeerden 212 MSM zonder diarree, 109 MSM die onlangs diarree hadden en 68 MSM die diarree meldden op de dag van het bezoek aan de kliniek. Dertien (3,3%, 95% CI 1,7-5,6%) MSM waren geïnfecteerd met *Shigella*, geen enkele met *Salmonella*, en 7 (1,8%, 95% CI 0,7-3,7%) met *Campylobacter*. De prevalentie van *Shigella* was 2,8% (95%CI 1,0-6,1%) bij asymptomatische mannen, 3,7% (95%CI 1,0-9,1%) bij mannen die recent diarree hadden en 4,4% (95%CI 0,9-12,4%) bij mannen met huidige diarree ($P=0,799$). *Shigella* werd vaker gevonden bij MSM die in de voorgaande drie maanden (10/151) PrEP hadden gebruikt dan bij mensen die geen PrEP hadden gebruikt (2/146) of hiv-positief waren (1/75) ($p=0,038$). *Shigella* werd significant vaker gedetecteerd bij MSM in vergelijking met routinematig verkregen fecale monsters, namelijk 11/770 (1,4%) ($p=0,031$).

Conclusie

Shigella infecties komen relatief vaak voor bij zowel symptomatische als asymptomatische MSM. Toekomstige studies moeten zich richten op het risico van verdere overdracht via asymptomatische personen.

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.¹ Not

all infections result in clinical symptoms and not all persons with clinical symptoms are diagnosed.¹ 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.^{2,3}

In many countries, including the Netherlands, isolation of *Shigella* is required for notification of shigellosis.^{4–6} The sensitivity of culturing is limited,⁷ but antimicrobial susceptibility testing is important, as *Shigella* species are developing resistance against several antibiotic groups.⁸ *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,⁹ 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).⁹ In a Danish study, MSM had a matched OR (mOR) of 105 of *Shigella* infections compared with controls.¹⁰ In a US study on gastroenteritis in MSM, 30% had shigellosis.¹¹ Several outbreaks of multidrug-resistant *Shigella* strains, such as with *S. flexneri* 3a in England, have been reported among MSM.^{12–14} 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.¹⁵

In the Netherlands in 2009–2016, 360–752 shigellosis cases yearly were confirmed and notified.² 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.¹⁶

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.¹⁷ In adults in high-income countries, no systematic studies regarding duration of *Shigella* carriership or *Shigella* PCR positivity have been done, although *Shigella* long-term carrier state has been described.¹⁸ 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.¹

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).¹⁹ However, this study did not focus on key populations like MSM. Little is known whether and how often asymptomatic *Shigella* infections occur in MSM.²⁰

Salmonella and *Campylobacter* have also been reported among MSM and to be sexually transmissible.^{21,22} 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%).¹¹ 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.¹⁰ 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.¹⁹ We assumed a prevalence of 2% *Shigella* among asymptomatic MSM. As a reference for the presence of shigellosis in MSM with diarrhoea, we used 30% as found in a study conducted in the USA¹¹ and divided this by two since we expect a lower prevalence in the Netherlands, resulting in an expected prevalence of 15% 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.²⁰ 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 a RotorGene (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,⁹ 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

Table 1 Primers and probes for *Shigella*/EIEC, *Salmonella* and *Campylobacter*

Micro-organism	Gene	Primer/probe	Sequence 5'–3'
<i>Shigella</i> /EIEC	<i>ipaH</i>	Forward	CCT TTT CCG CGT TCC TTG A
		Reverse	CGG AAT CCG GAG GTA TTG C
		Probe	ROX - CGCCTTCCGATACCGTCTCTGCA - BHQ2
<i>Salmonella</i>	<i>ttrC</i>	Forward	CTC ACC AGG AGA TTA CAA CAT GG
		Reverse	AGC TCA GAC CAA AAG TGA CCA TC
		Probe	JOE-CACCGACGGCGAGACCGACTTT-TAM
<i>Campylobacter jejuni</i>	<i>mapA</i>	Forward	CTG GTG GTT TTG AAG CAA AGA TT
		Reverse	CAA TAC CAG TGT CTA AAG TGC GTT TAT
		Probe	FAM - AAT TCC AAC ATC GCT AAT G
<i>Campylobacter coli</i>	<i>ceuE</i>	Forward	TCT CGC TTT GGA ATC ATT CAT G
		Reverse	CAT GTG TGC CTA CTT TTA CAT TTT CA
		Probe	FAM - TGT TTT AGG AAT CAA TGC TG

Table was created by the authors.
EIEC, entero-invasive *Escherichia coli*.

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 χ^2 test or Fisher's exact test, except for age, which was compared using the Kruskal-Wallis 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 χ^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.

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 23 were from MSM, 15 were travel related and for 10 none of these applied.

Salmonella and *Campylobacter* infections

We detected no *Salmonella* infections in the included MSM population. In comparison, in routinely tested faecal samples during the same study period, 3/771 (0.4%; 95% CI 0.1 to 1.1%) samples were positive for *Salmonella* ($p=0.555$). 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* ($p=0.468$).

Table 2 Characteristics of MSM clients visiting the STI clinic Amsterdam, March–June 2020, who were included in the study, by diarrhoea history

	Total (n=389)	No diarrhoea (n=212)	Diarrhoea last month (n=109)	Currently diarrhoea (n=68)	P value*
Age in years, median (IQR)	32 (26–44)	33 (26–46)	33 (26–44)	29 (26–37.5)	0.088
Age in years, n (%)					0.579
	16–24	67 (17.2)	37 (17.5)	17 (15.6)	
	25–34	147 (37.8)	77 (36.3)	39 (35.8)	
	35–44	78 (20.0)	40 (18.9)	26 (23.9)	
	45–75	97 (24.9)	58 (27.4)	27 (24.8)	
Country of origin, n (%)					0.034
	Netherlands	161 (41.4)	91 (42.9)	53 (48.6)	
	Other Europe	80 (20.6)	39 (18.4)	18 (16.5)	
	America	67 (17.2)	40 (18.9)	13 (11.9)	
	Africa	19 (4.9)	11 (5.2)	6 (5.5)	
	Asia and Oceania	61 (15.7)	31 (14.6)	18 (16.5)	
	Unknown	1 (0.3)	0	1 (0.9)	
HIV and PrEP status, n (%)					0.006
	HIV–, no PrEP use	146 (37.5)	81 (38.2)	40 (36.7)	
	HIV–, PrEP use	151 (38.8)	82 (38.7)	50 (45.9)	
	HIV+	75 (19.3)	42 (19.8)	11 (10.1)	
	Unknown	17 (4.4)	7 (3.3)	8 (7.3)	
No. of sex partners in last 6 months, n (%)					0.814
	0 or 1	35 (9.0)	20 (9.4)	10 (9.2)	
	2–4	96 (24.7)	52 (24.5)	25 (22.3)	
	5–9	88 (22.6)	53 (25.0)	21 (19.3)	
	≥10	168 (43.2)	86 (40.6)	53 (48.6)	
	Unknown	2 (0.5)	1 (0.5)	0	
Anal symptoms, n (%)					<0.001
	No	360 (92.5)	210 (99.1)	102 (93.6)	
	Yes	29 (7.5)	2 (0.9)	7 (6.4)	
	Discharge	20 (5.1)	1 (0.5)	2 (1.8)	
	Burning sensation	4 (1.0)	0	0	
	Wounds	4 (1.0)	0	4 (3.7)	
	Itch	1 (0.3)	0	1 (0.9)	
	Warts	1 (0.3)	1 (0.5)	0	
Timing of sample with regard to lockdown period					<0.001
	Before lockdown (until 16 March)	333 (85.6)	212 (100)	108 (99.1)	
	During lockdown	56 (14.4)	0	1 (0.9)	
NG†, n (%)					0.167
	Negative	339 (87.2)	183 (86.3)	100 (91.7)	
	Positive	50 (12.9)	29 (13.7)	9 (8.3)	
CT†, n (%)					0.606
	Negative	354 (91.0)	193 (91.0)	101 (92.7)	
	Positive	35 (9.0)	19 (9.0)	8 (7.3)	
Syphilis, n (%)					0.226
	Negative	384 (98.7)	211 (99.5)	106 (97.3)	
	Positive	5 (1.3)	1 (0.5)	3 (2.8)	
Hepatitis B, n (%)					0.276
	Negative	388 (99.7)	212 (100)	108 (99.1)	
	Positive	1 (0.3)	0	1 (0.9)	
<i>Shigella</i> , n (%)					0.799
	Negative	376 (96.7)	206 (97.2)	105 (96.3)	
	Positive	13 (3.3)	6 (2.8)	4 (3.7)	
<i>Campylobacter</i> , n (%)					0.215
	Negative	382 (98.2)	210 (99.1)	105 (96.3)	
	Positive	7 (1.8)	2 (0.9)	4 (3.7)	
SARS-CoV-2, n (%)					0.212
	Negative	385 (99.0)	211 (99.5)	108 (99.1)	
	Positive	4 (1.0)	1 (0.5)	1 (0.9)	

Table was created by the authors.

*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.

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 diarrhoea at clinic visit or in the last month.

No significant difference in *Shigella* prevalence was found between MSM with current or recent 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 asymptomatic. *Shigella*-positive samples were more frequently found in

Table 3 Associations between possible risk factors and *Shigella* infections among MSM routinely visiting the STI clinic Amsterdam, March–June 2020

		Shigella positive	P value*	OR	95% CI
Age in years, n (%)	16–24	1/67 (1.5)	0.808	1	
	25–34	6/147 (4.1)			
	35–44	2/78 (2.6)			
	45–75	4/97 (4.1)			
Country of origin, n (%)	Netherlands	8/161 (5.0)	0.134	1	
	Other	5/228 (2.2)			
HIV and PrEP status, n (%)	HIV–, no PrEP use	2/146 (1.4)	0.038	1	
	HIV–, PrEP use	10/151 (6.6)			
	HIV+	1/75 (1.3)			
	Unknown	0/17			
No. of sex partners in last 6 months, n (%)	0 or 1	2/35 (5.7)	0.332	1	
	2 or more	11/352 (3.1)			
	Unknown	0/2			
NG, n (%)	Negative	10/339 (3.0)	0.226	1	
	Positive	3/50 (6.0)			
CT, n (%)	Negative	12/354 (3.4)	1.000	1	
	Positive	1/35 (2.9)			
Syphilis, n (%)	Negative	12/384 (3.1)	1.000	1	
	Positive	1/5 (2.0)			
Hepatitis B, n (%)	Negative	13/388 (3.4)	1.000	N.A.	N.A.
	Positive	0/1			
<i>Campylobacter</i> , n (%)	Negative	13/382 (3.4)	1.000	N.A.	N.A.
	Positive	0/7			
SARS-CoV-2, n (%)	Negative	13/385 (3.4)	1.000	N.A.	N.A.
	Positive	0/4			
Anal symptoms, n (%)	None	12/360 (3.3)	1.000	1	
	Yes	1/29 (3.5)			

Results of univariable logistic regression. Table was created by the authors.
 *P values were determined excluding men with unknown values.
 CT, *Chlamydia trachomatis*; MSM, men having sex with men; NG, *Neisseria gonorrhoeae*; PrEP, pre-exposure prophylaxis.;

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 *Shigella* infection and the number of sexual partners. However, we discriminated only between 0–1 and 2 or more sexual partners in the last 6 months. The number of *Shigella* infections was too low to perform a more detailed analysis. Previously oro-anal contact was associated with transmission of *C. trachomatis*, *N. gonorrhoeae*²³ and extended-spectrum β -lactamase Enterobacteriaceae.²⁴ Oro-anal contact seems to be the main risk factor for acquiring *Shigella* infections in MSM.

Extrapolating from the prevalences of 2.8%, 3.7% and 4.4% of shigellosis among 212 asymptomatic, 109 recently symptomatic and 68 symptomatic MSM in our study population to the 2307 asymptomatic, 512 recently symptomatic and 110 symptomatic MSM that visited the clinic during the 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 201) 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.⁹

Previously, *Shigella* was detected in 0.9% of patients with diarrhoea in the Netherlands but not detected in healthy controls.¹⁹ 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.¹⁹ In a Danish study, *Campylobacter* and *Shigella* infections were associated with being MSM, but *Salmonella* infection was not.¹⁰ 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 notifiable 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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Twitter Henry J.C. de Vries @henryjdevries

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ORCID iDs

Joyce F. Braam <http://orcid.org/0000-0002-5043-3424>

Sylvia M. Bruisten <http://orcid.org/0000-0003-4897-4261>

Henry J.C. de Vries <http://orcid.org/0000-0001-9784-547X>

Maarten F. Schim van der Loeff <http://orcid.org/0000-0002-4903-7002>

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